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# XRCC2 Gene Study by Next Generation Sequencing and Establishing Its Relation with Breast Cancer

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**Abstract**: Breast cancer (BC) is a leading cause of morbidity and mortality among women, with its development influenced by genetic factors such as mutations in the XRCC2 gene, a key player in DNA repair via homologous recombination. This study aimed to elucidate the role of XRCC2 in BC by utilizing Next Generation Sequencing (NGS) to identify genetic variants and assess their association with BC risk and progression. This study was done between 2021 and 2024. Specimens were meticulously collected from Zheen International Hospital, located in Erbil, Iraq. In total, 44 peripheral blood samples of 44 BC patients were included in this study. DNA extracted from these samples underwent NGS, revealing seven XRCC2 variants with varying predictions of pathogenicity. In terms of pathogenicity, 5 of these mutations were Uncertain Significance, including (c.134A>C, c.271C>T, c.283A>C, c.181C>A, c.-1G>A (5UTR variant)), 1 of them was Likely Pathogenic including (c.651_652del) and other (c.582G>T) was Likely benign. In conclusion, the XRCC2 could serve as a biomarker for BC, warranting further investigation for its inclusion in genetic screening programs.

**Keywords:** Breast cancer, Genomic analysis, Next generation sequencing

## Introduction

### Introduction to XRCC2 and Breast Cancer

Breast cancer (BC) is the most frequent malignancy among women worldwide, with approximately 2.3 million new cases identified annually (Arnold et al., 2022). BC is the most commonly diagnosed cancer among women worldwide, with a multifactorial etiology that encompasses genetic, environmental, and lifestyle factors (Obeagu and Obeagu, 2024). Among the myriad of genes implicated in the maintenance of genomic integrity, the X-ray repair cross-complementing group 2 (XRCC2) gene is a pivotal element in the homologous recombination repair (HRR) pathway (Liu et al., 2023, Yu & Wang, 2023).

The XRCC2 gene plays a pivotal role in the HRR pathway, a crucial mechanism for the repair of DNA double-strand breaks (DSBs) (Yu and Wang, 2023). DSBs are among the most lethal forms of DNA damage, and if not accurately repaired, they can lead to genomic instability, a hallmark of cancer development (Alhmoud et al., 2020, Berzsenyi et al., 2021). The XRCC2 gene provides a plugin for the formation of the RAD51 paralog complex that is vital for the processing of DSBs and the maintenance of chromosome stability Mutations or dysregulation in the components of the HRR pathway, including XRCC2, can compromise DNA repair and contribute to carcinogenesis (Ivy et al., 2021, Yu &Wang, 2023). The XRCC2 gene, by virtue of its role in HRR, has thus become a subject of intense research interest, particularly in the context of BC susceptibility (Liu et al., 2021).

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**Next Generation Sequencing (NGS) and Its Role in XRCC2 Analysis**

The evolution of NGS technologies has tremendously changed the way genetic research is conducted as it allows full-scale analysis of genomic sequences with unmatched detail and extent. NGS offers a detailed and efficient approach to identifying genetic variations, including single nucleotide polymorphisms (SNPs), insertions, deletions, and complex rearrangements (Satam et al., 2023). These variations, when occurring within or in close proximity to genes like XRCC2, can potentially modulate gene function and, consequently, an individual's risk of developing BC (Yu &Wang, 2023). By employing NGS, researchers can dissect the intricate relationship between XRCC2 gene variants and BC predisposition with greater specificity and sensitivity than was previously possible with traditional sequencing methods (Qin, 2019).

**Necessity and Aim of the Present Study**

Despite the progress made in understanding the genetic landscape of BC, the role of XRCC2 in BC susceptibility remains incompletely characterized. Previous studies have provided conflicting results regarding the association of XRCC2 variants with BC risk, necessitating further investigation using large-scale, robust genomic methodologies like NGS.

A study conducted by Dastgheib et al. (2024) showed that XRCC2 may play a role in increasing the risk of BC (Dastgheib et al., 2024). On the other hand, a discovery by Decker et al. (2017), showed that rare, protein-truncating variants in ATM, CHEK2, or PALB2, but not XRCC2 were associated with increased BC risks (Decker et al., 2017). The present study is therefore essential to clarify the contribution of XRCC2 to BC and to establish whether it should be considered as part of genetic screening programs for at-risk populations.

## Method

**Specimen Collection and Ethical Considerations**

In this observational analytical research study that utilizes a case-control design, we focused on the analysis of the XRCC2 gene and its association with BC. This study was done between 2021 and 2024. Specimens include 44 peripheral blood samples with BC were collected from Zheen International Hospital, located in Erbil, Iraq. These samples were stratified based on the type of BC, patient age, and clinical characteristics.

All subjects provided informed permission before sample collection, and the Local Ethics Committee accepted the study procedure (Approval number: 05.01.2020\17). To ensure the integrity of the RNA, tissues were preserved in RNALater (ThermoFisher, USA) until genomic isolation could be performed.

**DNA Extraction and Quantification**

Following the manufacturer's instructions, DNA was extracted from peripheral blood samples using the PureLinkTM genomic DNA micro kit (ThermoFisher, USA). Using a NanoDrop (Biometrica-Taiwan), the concentration and purity of DNA were measured.

**Next Generation Sequencing (NGS)**

Using the Twist Human Core Exome Enzymatic Fragmentation (EF) Multiplex Complete kit, libraries were prepared for next-generation sequencing (NGS). Next, we used the MGIEasy FS DNA Library Prep Kit to get this library ready for sequencing. A mean target coverage of 100X was attained by sequencing the prepared library using the MGI-DNBSEQ-G400 platform (China), which produced 150 bp paired-end reads. FastQC was used for quality control on the raw FASTQ files. Burrows-Wheeler Aligner (BWA) was used to align reads to the human reference genome (hg19), and the Genome Analysis Toolkit (GATK) program was used to call variants. The depiction of variants was done using the Integrative Genomic Viewer (IGV).

**In Silico Analysis**

To estimate the effect of mutations on protein function, in silico methods like Sorting Intolerant from Tolerant (SIFT) and Polymorphism Phenotyping (PolyPhen-2) were used. Mutation Taster and Align GVGD were used to evaluate the effects of mutations on protein structure and function.

**Primer Design and PCR Optimization**

Primers targeting the XRCC2 gene were designed to span exon-exon junctions, ensuring specificity to mRNA. Primers for the XRCC2 gene and the housekeeping gene GAPDH were designed using an online primer design program (http://workbench.sdsc.edu). The PCR conditions were optimized using gradient thermocycling in an ABI Vertti PCR System (ABI, USA). The optimal annealing temperature was determined by examining the yield of PCR products on a 2% agarose gel (Table 1).

Table 1. Primer sequences, PCR product size of three targets region of XRCC2/Exp optimal annealing temperature.

| Gene name | | Primer sequence | Optimal annealing temperature | PCR Product size |
|---|---|---|---|---|
| XRCC2 | F<br>R | TGTTTGCTGATGAAGATTCAC<br>TCGTGCTGTTAGGTGATAAAGC | 59.2 °C | 255 bp |
| GAPDH | F<br>R | GGTCCACCACCCTGTTGCTGT<br>AGACCACAGTCGATGCCATCAC | 59,4 °C | 456 bp |

## Results and Discussion

To elucidate the implications of germline variants in breast cancer, seven inherited mutations identified within the XRCC2 gene were subjected to in silico analysis using a suite of computational prediction tools. The functional consequences of these variants were evaluated using Polymorphism Phenotyping (PolyPhen), which assesses potential structural and functional changes due to amino acid substitutions. PolyPhen scores provide a gradient of functional impact, ranging from benign (score of 0.0), possibly damaging (score between 0.15 and 0.85), to likely damaging (score above 0.85). Additionally, the Sorting Intolerant from Tolerant (SIFT) algorithm was employed, which examines sequence homology and the conservation of amino acid residues to predict the phenotypic effects of substitutions, with scores below 0.05 indicating deleterious changes.

MutationTaster and Align-GVGD further supplemented the predictive landscape, with the former analyzing potential impacts on protein function, mRNA expression, or splicing, and categorizing mutations from benign polymorphisms to likely disease-causing alterations. Align-GVGD combines biochemical distance scores (Grantham difference) and conservation scores (Grantham variation) to stratify substitutions into seven classes, with C0 being the least likely to affect the function and C65 the most.

Table 2. XRCC2 mutations identified in BC patients.

| SNP ID | Allele Change | Amino Acid Change | Molecular consequence | Interpretation | SIFT Prediction | PolyPhen Prediction |
|---|---|---|---|---|---|---|
| * | c.134A>C | p.Glu45Gly | missense | Uncertain significance | Deleterious | NA |
| rs730882043 | c.271C>T | p.Arg91Trp | missense | Uncertain significance | Deleterious | Probably Damaging |
| rs140214637 | c.283A>C | p.Ile95Val | missense | Uncertain significance | Tolerated | Benign |
| rs746142129 | c.651_652 del | p.Cys217_Asp 218delinsTer | frameshift | Likely pathogenic | NA | NA |
| rs769829135 | c.582G>T | p.Thr194= | synonymous | Likely pathogenic | NA | NA |
| rs569810249 | c.181C>A | p.Leu61Ile | missense | Uncertain significance | Deleterious | Possibly Damaging |
| rs768232997 | c.-1G>A | - | 5 prime UTR | Uncertain significance | NA | NA |

* This mutation was observed for the first time in this study in BC

The present study investigated the mutations created in the XRCC2 gene in 44 BC patients by the NGS method. After performing this method, 7 mutations were found in the mentioned gene. All these mutations were heterozygous. In terms of pathogenicity, 5 of these mutations were Uncertain Significance, including (c.134A>C, c.271C>T, c.283A>C, c.181C>A, c.-1G>A (5UTR variant)), 1 of them was Likely Pathogenic including (c.651_652del) and other (c.582G>T) was Likely benign (Table 2).

The XRCC2 gene that encodes a protein related to DNA repair has been examined in BC. The XRCC2 gene is one component of the RAD51 gene family that has seven primary copies maintained and is involved in homologous recombination as well as DNA repair (Wang et al., 2014, Kluźniak et al., 2019). High-throughput sequencing technology known as NGS has completely changed the fields of molecular biology and genomics. Large volumes of DNA or RNA can be quickly and accurately sequenced thanks to it, allowing researchers to examine intricate genetic and biological systems in previously unheard-of detail (Satam et al., 2023). Therefore, this study aimed to elucidate the role of XRCC2 in BC by utilizing NGS to identify genetic variants and assess their association with BC risk and progression.

The present study using Next-Generation Sequencing (NGS) identified 7 heterozygous mutations in the XRCC2 gene, with varying degrees of pathogenicity. Five mutations were classified as Variants of Uncertain Significance (VUS), one as Likely Pathogenic, and one as Likely Benign. The mutation c.134A>C, known as p.Glu45Gly, in the XRCC2 gene's coding exon 3 is of uncertain significance. This variant that was observed for the first time in this study in BC involves an A to C substitution at nucleotide position 134, leading to the replacement of glutamic acid with glycine, an amino acid with different properties. In-silico tests indicate that this missense mutation at a conserved and deleterious amino acid position is associated with hereditary cancer-predisposing syndromes. Due to the significant changes it induces in the protein's amino acid sequence, it is likely to disrupt the protein's function.

Another mutation, XRCC2 c.271C>T, causes the p.Arg91Trp (R91W) to change from Arginine to Tryptophan at the protein level. This alteration, observed in BC families, shows potential protein structure and function damage as per in silico analyses. It has been identified in Caucasian families with a strong BC history and has shown a moderate ability to restore XRCC2-DNA repair deficiencies in certain complementation assays (Hilbers et al., 2012, Park et al., 2012). While this alteration has been detected in a BC patient within a UK study, its clinical significance remains uncertain due to limited supporting evidence and inconclusive in-silico predictions (Kluźniak et al., 2019). The position of this altered amino acid is highly conserved in vertebrate species, adding to the complexity of determining its clinical impact.

The c.582G>T variant in ClinVar database is considered likely pathogenic despite being a synonymous mutation with a low population frequency (1 in 100,000). The XRCC2 protein's c.181C>A variant results in a conservative p.Leu61Ile substitution, with leucine being replaced by isoleucine (both neutral, non-polar amino acids). Despite its presence in population databases (rs569810249, gnomAD 0.02%) and association with certain cancers (BC and stomach cancer) (Park et al., 2012, Lu et al., 2015), experimental studies suggest minimal impact on XRCC2 function (Hilbers et al., 2016). The variation occurs at a conserved position but not within a recognized functional domain, leading to mixed predictions about its influence on protein structure and function. Finally, the c.-1G>A variant in the XRCC2 gene's 5' UTR shows a G to A substitution close to the translation starting site. Its occurrence in a small subset of early-onset BC patients versus controls, and conservation across vertebrates, suggests potential significance, yet clinical relevance remains uncertain due to limited evidence (Park et al., 2012).

Given the significant associations observed between XRCC2 variants and BC, this gene merits consideration for inclusion in genetic screening programs for at-risk populations. Such screening could potentially aid in early detection and personalized therapeutic strategies, improving patient outcomes.

## Conclusion

Through Next-Generation Sequencing, we identified seven heterozygous mutations in the XRCC2 gene, with one being likely pathogenic, one likely benign, and five of uncertain significance. The potential impact of these mutations on protein function varies, with some showing a possible link to cancer predisposition. These findings highlight the XRCC2 gene's role in the DNA repair process and underscore the importance of including it in genetic screening for breast cancer, which could lead to enhanced early detection and more individualized treatments for patients at risk. Further research is needed to clarify the clinical significance of the uncertain variants and to determine their precise role in breast cancer etiology.

## Recommendations

Future studies should look at increasing the size of the cohort so that the guided XRCC2 mutations can be clinically validated. Moreover, they should be explored as potential biomarkers for both the BC risk and prognosis. Presence or absence of these mutation may be asymmetrical, which may give rise to health conditions and that needs to be examined further. Development and implementation of the XRCC2 genetic screening could be thought of in the high-risk populations; it should be combined with the creation of individual treatment regimens depending on the status of this gene. Subsequently, research is needed to investigate the role of XRCC2 in therapy resistance and as a key point for new therapeutic methods.

## Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

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