Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Association of XRCC2 with breast cancer, a multi-omics analysis at genomic, transcriptomic, and epigenomic level

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Article Info

Article history:

Received: March 31, 2024
Accepted: May 05, 2024
Published: July 31, 2024

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Abstract

One of the main causes of cancer-related mortality for women worldwide is breast cancer (BC). The XRCC2 gene, essential for DNA repair, has been implicated in cancer susceptibility. This study aims to evaluate the association between XRCC2 and BC risk. The study was conducted at Zheen International Hospital in Erbil, Iraq, between 2021 and 2024 with a total of 88 samples, including 44 paired normal and cancer tissue samples. Mutation analysis was performed using Next-Generation Sequencing, coupled with in silico tools for variant impact prediction. Expression levels were assessed through RT-PCR, and methylation status was determined using methylation-sensitive restriction enzyme digestion PCR. The study identified seven inherited germline variants in the XRCC2 gene, with five of these mutations being Uncertain Significance, one being Likely Pathogenic, and one being Likely benign. RNA purity was found high with mean A260/280 ratios of 1.986 ± 0.097 in normal (N) and 1.963 ± 0.092 in tumor (T) samples. Tumor samples exhibited a higher RNA concentration (78.56 ± 40.87 ng/µL) than normal samples (71.44 ± 40.79 ng/µL). XRCC2 gene expression was significantly upregulated in tumor tissue, with marked increases in patients aged 40-55 and >56 years and in higher cancer grades (II and III) and invasive ductal carcinoma (p-values ranging from <0.0001 to 0.0392). DNA methylation rates in tumor tissues were low (7%), suggesting limited regulation by methylation. The study suggests that XRCC2 can be classified as an oncogene and that its structural investigation by targeted NGS and expression evaluation can be used as a potential biomarker in BC.

Keywords: Breast Cancer, DNA Methylation, Genomic Analysis, Next-Generation Sequencing

1. Introduction

Breast cancer (BC) is a complex and multifaceted disease that affects millions of women worldwide [1]. Based on GLOBOCAN 2020 statistics, the number of new cases of BC is predicted to reach 2.3 million worldwide, ranking it as the fifth most prevalent cause of cancer-related fatalities and one of the most frequently diagnosed malignancies [2].

The causes of BC are multifactorial and include genetic, hormonal, and lifestyle factors. Some risk factors include family history, age, reproductive history, hormonal exposure, and certain lifestyle choices [3, 4]. Patients with BC may be asymptomatic in the early stages or present with clinical manifestations such as a palpable lump, changes in breast shape or size, skin dimpling, nipple retraction, or discharge. As the disease progresses, local invasion and distant metastasis may lead to myriad complications, including pain, organ dysfunction, and ultimately, life-threatening consequences, highlighting the importance of regular screening and early detection [5, 6].

Diagnosis of BC typically involves a combination of clinical assessment, imaging techniques like mammography and ultrasound, and histopathological evaluation through biopsy [7]. The discovery of certain genetic changes linked to BC has also been made possible by advances in molecular biology, and this information can help with prognosis, diagnosis, and personalized therapy [8, 9].

The pathophysiology of BC is characterized by the transformation of normal breast epithelial cells into malignant ones, a process driven by genetic mutations and various signaling pathways [10]. Among the multitude of genes implicated in BC, XRCC2 has garnered attention for its role in DNA repair [11]. A protein called XRCC2 has been linked to the risk of BC. It is involved in both DNA double-strand break repair and homologous recombination (HR). As part of the HR repair mechanism, XRCC2 is crucial for maintaining genomic stability. Mutations in the XRCC2 gene may compromise DNA repair, leading to genetic aberrations that can initiate or promote carcinogenesis [12].

The XRCC2 gene polymorphisms may influence the risk of BC, according to research by Wang et al. (2023), which also found that certain SNPs and haplotypes in XRCC2 are connected to BC traits in the Han population of northwest China [13]. On the contrary, according to Wojciech Kluśniak et al.'s [14] study, there is no proof that this mutation puts people at risk for BC or other malignancies. XRCC2 should not be regarded as a gene that predis-

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Doi: http://dx.doi.org/10.14715/cmb/2024.70.7.36

poses people to BC.

Given this backdrop, the necessity of conducting comprehensive research on the XRCC2 gene's association with BC becomes apparent and valuable. The present study aims to bridge the gap in knowledge by examining the relationship between XRCC2 and BC not merely at the genomic level but extending the inquiry to transcriptomic and epigenetic dimensions. This will update the knowledge of the relationship between the XRCC2 gene and BC.

2. Materials and Methods

2.1. Study Design and Participants

The present study was conducted at Zheen International Hospital in Erbil, Iraq and the Department of Biology, Gaziantep University between 2021 and 2024. The study was performed on 88 samples in total, 44 of which were paired samples of normal and cancerous tissue. The tissue samples were categorized based on the patient's age, clinical features, and type of BC.

The inclusion criteria for the study were adult females aged 18 or above with a histologically confirmed diagnosis of BC. Exclusion criteria included patients with a history of other malignancies, those receiving chemotherapy or radiation therapy before sample collection, or those with incomplete medical records. The sample size was determined based on a power analysis to detect a minimum clinically significant difference in expression levels with a power of 80% and an alpha of 0.05.

2.2. Mutation analysis

2.2.1. DNA extraction

Following the manufacturer's instructions, DNA was isolated from peripheral blood and FFEP samples using the PureLinkTM genomic DNA micro kit (ThermoFisher, USA). Using NanoDrop (Biometrica-Taiwan), DNA concentration was quantified and qualified.

2.2.2. Next-generation sequencing (NGS)

The Twist Human Core Exome Enzymatic Fragmentation (EF) Multiplex Complete kit was utilized for the construction of the library, while the MGIEasy FS DNA Library Prep Kit was employed to prepare the library for sequencing on the MGI system. Sequencing of the library was carried out on the (MGI-DNBSEQ-G400, China) instrument, producing 150 bp paired-end reads with a mean target coverage of 100X. Quality control of Raw fastq files was performed using FastQC. Subsequently, the reads were mapped to the reference human genome (hg19) using the Burrows-Wheeler Aligner (BWA). Variants were detected using the Genome Analysis Toolkit (GATK). Visualization of the variants was facilitated through the use of Integrative Genomic Viewer software (IGV).

2.3. In silico analysis

To forecast the impact of mutation on the structural characteristics or protein function, several in silico approaches were employed. The functional impacts of variations were evaluated using Polymorphism Phenotyping (PolyPhen-2) [15] and Sorting Intolerant from Tolerant (SIFT) [16]. To assess the impact of mutations on the structure and function of proteins, the Mutation Taster was employed. A biochemical distance score was calculated using Align Grantham Variation Grantham Deviation (GVGD) [17].

2.4. Expression analysis

2.4.1. RNA extraction and complementary DNA synthesis

The RNA extraction was performed using the extraction kit from Thermofisher-USA, with Nano Drope (Biometrica, Taiwan) used to quantify and qualify the total RNA concentration. The Ipsogen RT Kit (Qiagen, GmbH, Hilden, Germany) was used for complementary DNA (cDNA) synthesis, with the Master-cycler pro-PCR System (Eppendorf, Germany) used in the thermal cycling process. Ethanol 70% (v / v) and filter tips were used to clean the workspace and ensure sterility in all steps. Given that the samples' total RNA concentration and quality varied, it was advised to do a control reaction devoid of reverse transcriptase to look into any potential DNA interference.

The components of the system were thawed and put on ice for DNA synthesis. To check for DNA contamination, a control reaction devoid of reverse transcriptase was advised. The RNA sample and primer d(T)23 VN were mixed in sterile RNase-free tubes, with total RNA varying from 1 to 6 µl and d(T)23VN (50 µM) added in 2 µl. The RNA was then denatured at 70 °C for 5 min, followed by incubation at 42 °C for 1 hour. The enzyme was inactivated at 80 °C, and the reaction was diluted to 50 µl for PCR. The volume of the PCR reaction should not be more than 1/10 of the cDNA output.

2.4.2. Primer design

The primer design involved a pair of mRNA sequence primers for XRCC2/Exp, with the online primer design program http://workbench.sdsc.edu employed. The primer sequences, annealing temperature, and PCR product size are provided in Table 1. The primers were engineered to encompass the complete coding sequence of the gene, incorporating one or two exon-exon junction regions to inhibit the amplification of any other product save the RNA product in question.

2.4.3. PCR Optimization

In optimizing PCR conditions for specific cDNA primers, a gradient PCR was conducted using an ABI Vertti PCR System. The optimal annealing temperature was determined by analyzing the yield on an agarose gel, which was found to be 59.2°C for the XRCC2 primers. The PCR mixture for a total volume of 25µL contained 15µL of

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	TGTTTGCTGATGAAGATTCAC	59.2 °C	255 bp
	R	TCGTGCTGTTAGGTGATAAAGC		
GAPDH	F	GGTCCACCACCCTGTTGCTGT	59,4 °C	456 bp
	R	AGACCACAGTCGATGCCATCAC		

dH2O, 2.75µL of 10X PCR buffer with ammonium sulfate, 2µL of 25 mM MgCl2, 1.5µL of 2 mM dNTP mix, 1µL each of 20 mM forward and reverse primers, 0.125µL of 5 U/mL Taq DNA polymerase, and 1.5µL of cDNA template.

The thermocycling conditions included a 7 min initial denaturation at 94°C, followed by 40 cycles of 94°C for 40 sec, annealing between 55°C and 60°C for 40 sec and extension at 72°C for 40 seconds, concluding with a final extension at 72°C for 5 min and then holding the reaction at 4°C. Post-PCR, the products were assessed through 2% agarose gel electrophoresis stained with ethidium bromide, run at 100 volts for 60 minutes, and visualized under UV light.

2.4.4. GAPDH amplification

For normalization in gene expression studies, GAPDH, a housekeeping gene, was utilized to quantify mRNA levels of the target gene XRCC2, given that housekeeping genes are expected to have stable expression across different conditions and samples.

2.4.5. Real-time PCR

Real-time PCR was performed on a RotorGene 5 plex system using RT² SYBR Green ROX FAST Mastermix. The reaction mix for assessing XRCC2 expression was prepared with 10.5µL of the master mix, 1µL each of forward and reverse primers at 10 µM, 15.5µL of RNAse/DNAse free water, and 2.5µL of cDNA (50 ng), totaling a reaction volume of 30µL. The Real-Time PCR protocol involved an enzyme activation at 94°C for 10 min, followed by 40 cycles of denaturation at 94°C for 10 sec, primer annealing at the optimized 59.2°C for 40 sec, and extension at 72°C for 40 sec.

2.5. Statistical analysis

Quantification to Three RT-PCR runs was performed, producing data for XRCC2 expressed as the threshold cycle (Ct), which were then normalized using the housekeeping gene. The relative changes in XRCC2 expressions in tumor and normal samples were assessed separately using the $2^{-\Delta Ct}$ method about the housekeeping gene or internal control. The formula $2^{-\Delta\Delta Ct}$ was employed for statistical comparison of tissues based on the findings of qRT-PCR. For statistical analysis, SPSS 22.0 software (SPSS, Chicago, USA) was utilized. Student's t-test was used to compare two groups with normal distribution characteristics in order to evaluate the study data. A significance level of $p < 0.05$ was used for statistical analysis.

2.6. Methylation analysis

Based on the digestion of genomic DNA using a methylation-sensitive restriction enzyme and PCR using gene-specific primers, a methylation-sensitive restriction enzyme digestion PCR (MSRE-PCR) was used to analyze the XRCC2 promoter region's DNA methylation. Thermo Scientific EpiJET Methylation Analysis Kit (MspI/HpaII) instructions were followed in determining the DNA methylation status at a particular location. After overnight 1 µg DNA digestion was made possible by methylation-sensitive restriction enzyme cleavage by the isoschizomers Epi MspI and Epi HpaII, which have varying methylation sensitivity. Gene-specific primers were then used to amplify genomic fragments found within CpG islands in the amplified samples created by PCR. Primer design was performed for gene XRCC2 using Primer3 software, and primer sequences were as follows: XRCC2-M F-5'-TTGCTGCCATGCCTTACAGA-3', R-5'- TGGATAGACCGCGTCAA-3'. The formula for calculating the percentage of methylation was followed by the manufacturer. To identify a sample as methylated, a 20% cutoff was applied.

2.7. Ethical Approval

The Declaration of Helsinki was followed in the conduct of this study, and the local ethics committee (the ethical committee of Zheen Hospital) approved it after participants provided informed permission. Approval number: 05.01.2020\17.

3. Results

A total RNA quality and concentration assessment was conducted for samples from 44 BC patients. All samples were evaluated for purity using A260/280 ratios, with values around 2.0 being indicative of high purity. RNA concentrations varied across samples, with tumor samples generally exhibiting higher concentrations than normal samples, suggesting variability in RNA extraction efficiency or cellular RNA content (Table 2).

The present study of BC patients identified seven inherited germline variants in the XRCC2 gene. Utilizing in silico predictive tools, including PolyPhen, SIFT, and MutationTaster, assessed the functional implications of these variants. PolyPhen classified one variant as possibly damaging and another as probably damaging, whereas SIFT indicated that one variant was tolerated and others were deleterious. MutationTaster's predictions ranged from polymorphism to disease-causing mutations within the XRCC2 variants. 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 3).

The expression level was assessed using RT-qPCR. Comparing tumor samples to normal tissue samples, it was discovered that the XRCC2 gene was much more expressed (up-regulated) in the tumor samples (<0.0001, T-test; $p > 0.05$) (Figure 1).

A particularly pronounced increase was observed in

Table 2. Total RNA measurement and quality.

Variables	Group	Min-Max	Mean ± SD
Concentration ng/µL	N	13.60 - 233.50	71.44 ± 40.79
	T	19.50 - 202.40	78.56 ± 40.87
A260/280	N	1.70 – 2.20	1.986 ± 0.097
	T	1.80 – 2.20	1.963 ± 0.092

Table 3. 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_652del	p.Cys217_Asp218delinsTer	frameshift: stop-gain	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 and reported for the first time in this study in BC.

Table 4. Statistical significance according to age, cancer grade and types.

Variables	NO. (%)	XRCC2 Expression (p-value)	Mean of differences	SD of differences	SEM of differences
Age					
<40 years	13 (29.54)	0.8149	0.050	0.7832	0.2093
40- 55 Years	20 (45.45)	0.0392	0.0280	0.5653	0.1264
> 56 years	11 (20.45)	0.0191	0.3200	0.3553	0.1123
Cancer Grade					
I	9 (20.45)	0.8273	-0.06667	0.8874	0.2958
II	18 (40.9)	0.0013	0.3722	0.4099	0.09661
III	17 (38.63)	0.0051	0.2412	0.5789	0.1404
Breast cancer type					
Invasive ductal carcinoma	22 (50)	0.0006	0.3682	0.4247	0.09055
Carcinoma Medullary like	10 (22.72)	0.8088	0.0500	0.6346	0.2007
Matrix producing metaplastic	12 (27.27)	0.6647	0.1083	0.8426	0.2432

SEM: Standard error of means.

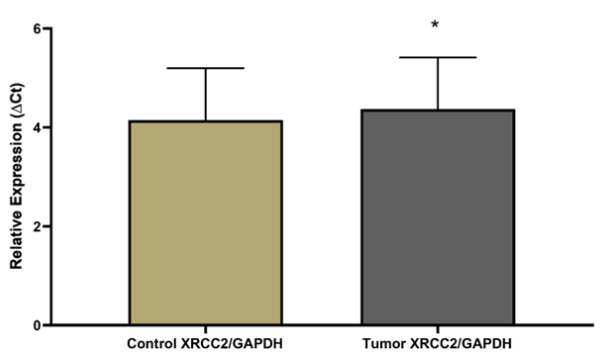

Fig. 1. The level of expression of mRNA.

patients aged 40-55 and over 56, suggesting age-related upregulation ($p < 0.05$). Furthermore, in BC grades II and III, the expression levels were significantly increased ($p < 0.05$), and notably in cases of invasive ductal carcinoma ($p < 0.05$). However, no significant variation was found in carcinoma medullary-like and matrix-producing metaplastic types ($p > 0.05$) (Table 4).

In terms of DNA methylation, the analysis indicated a low overall methylation rate of 7% in the XRCC2 gene in tumor tissues, with no evidence of promoter methylation. This implies that methylation has a restricted function in controlling the expression of XRCC2 in the examined tumor tissues (Figures 2 and 3).

The network analysis conducted using GeneMANIA revealed intricate gene-gene and protein-protein interactions for XRCC2, suggesting its multifaceted role in cellular processes pertinent to BC (Figure 4).

4. Discussion

Globally, BC is a major health problem, especially for

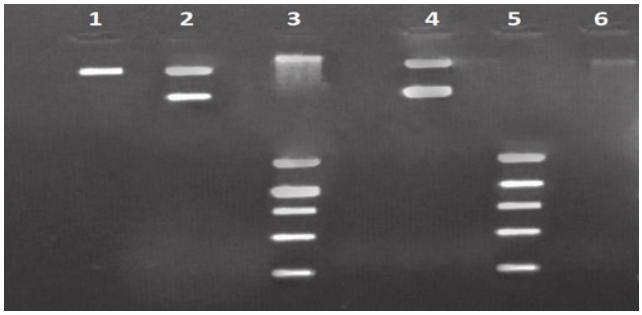

Fig. 2. Digestion of genomic and control DNA by Epi MspI and Epi HpaI. 1) Genomic DNA undigested; 2) contain Control pUC19/SmaI DNA CpG Methylated by Epi HpaI; 3) contain Control pUC19/SmaI DNA CpG Methylated by Epi MspI; 4) Genomic DNA and plasmid control undigested; 5) contain Control pUC19/SmaI DNA Unmethylated by Epi MspI; 6) Genomic DNA undigested.

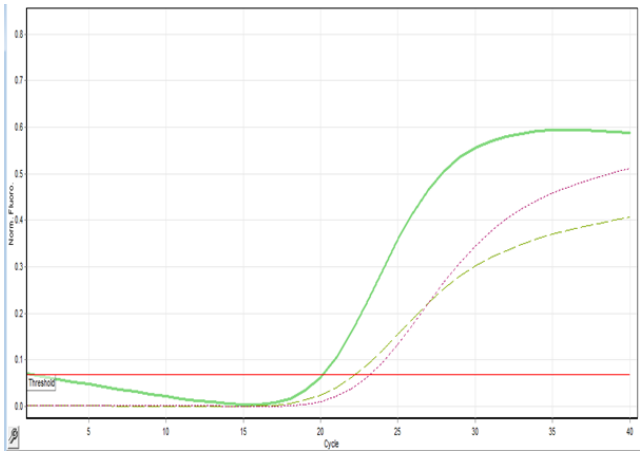

Fig. 3. Quantitative XRCC2 methylation status. The Green curve is "Undigested DNA", the Doted line is "Digested with Epi HpaII" DNA, and the dashed line is Digested with Epi MspI" DNA. The ct difference between the sample digested with MspI and HpaII was calculated to be significant and less than 4.7 (partially methylated). Finally, using the formula, 7% of the genes were found to be methylated.

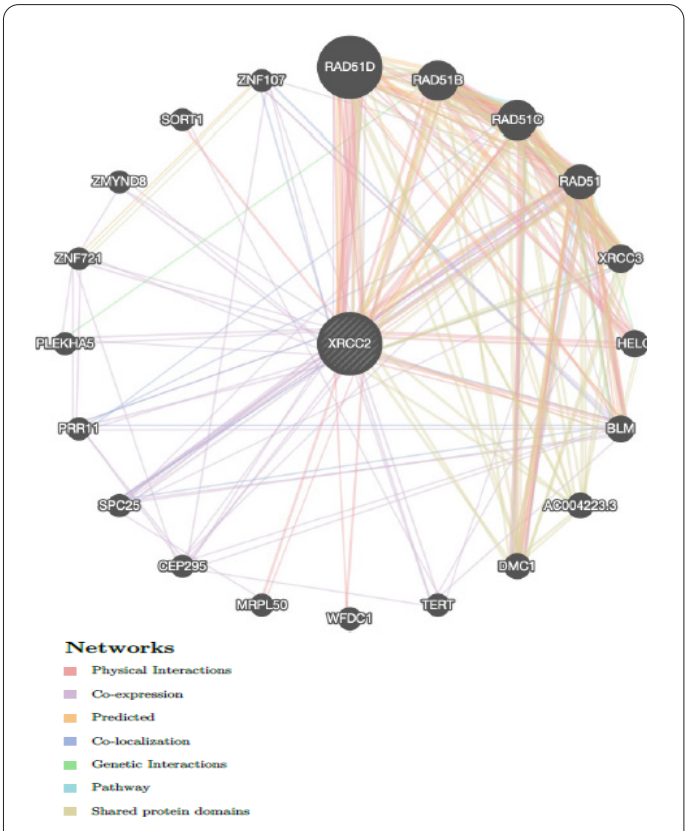

Fig. 4. The network of intricate gene-gene and protein-protein interactions for XRCC2.

women. It is one of the main causes of mortality worldwide and the most frequent malignancy among women. Race and ethnicity have a significant impact on the incidence and death rates of BC, with industrialized nations having higher rates [1]. Multiple genetic mutations have been identified as strongly correlated with an elevated susceptibility to BC. The XRCC2 gene is associated with BC risk. A study conducted by Gupta *et al.* [18] found that the Arg188His polymorphism of XRCC2 was associated with a reduced risk of BC. This study observed a positive interaction between XRCC3 and XRCC2 in BC risk. The present study was carried out to explore the association between XRCC2 and BC in Iraq at the genomic, transcriptomic, and epigenomic levels because there have been few investigations on this relationship, particularly multi-omics studies.

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. These alterations could explain the relation of XRCC2 gene and BC from genomic point of view.

Mutation c.134A>C is one of the uncertain significance variants found in this population. The XRCC2 gene's coding exon 3 has the p.Glu45Gly variation, sometimes referred to as c.134A>C. This variant is caused by an A to C substitution at nucleotide position 134. An amino acid with different characteristics, glycine, takes the place of glutamic acid at codon 45. In-silico test findings indicated that this amino acid position is harmful and conserved. This variant is missense and is related to conditions such as hereditary cancer-predisposing syndrome, Neoplastic Syndromes, Hereditary cancer-predisposing syndrome, Tumor predisposition, etc. This mutation was reported for the first time in this gene, due to its missense nature and the fundamental changes it creates in the amino acid sequence of the protein, it can be expected that the function of this protein will be disrupted.

The occurrence of a different genetic variation is identified as XRCC2 c.271C>T in the cDNA sequence, leading to the substitution of Arginine with Tryptophan (CGG>TGG) at the protein level denoted as p.Arg91Trp (R91W). While this specific mutation has been detected in families with BC history, it was not consistently passed down within one particular family [19]. According to in silico calculations, protein structure and function will most likely be harmed by this variation. Two Caucasian families with a notable history of BC have reported seeing this change [19, 20]. This alteration was also shown to have a moderate ability to restore XRCC2-DNA repair deficient phenotypes based on its performance in two out of three complementation assays [20]. This change has been documented in at least one BC patient in a UK research with

13087 BC patients and 5488 control subjects[14]. Several vertebrate species share this highly conserved amino acid location. Furthermore, there is conflicting silico prediction for this modification. The therapeutic relevance of this modification is not yet known, as there is a lack of supporting evidence.

Another variation linked to BC was c.283A>C, which is characterized by the replacement of the amino acid valine (p.Ile95Val) with the amino acid isoleucine at position 95. In the study conducted by Park et al. this mutation was found in a sample of male BC. According to this study, this mutation is a missense mutation that is generally benign [19].

A translational frameshift with a projected alternative stop codon (p.C217*) is caused by the deletion of two nucleotides at nucleotide locations 651 to 652 in the c.651_652delTG variation, which is found in coding exon 3 of the XRCC2 gene. This frameshift affects just the last 64 amino acids of the protein and happens at the 3' terminus of XRCC2. It is not anticipated to cause nonsense-mediated mRNA degradation. Functional studies using c-DNA complementation assays demonstrate that this alteration results in a partial loss (31% of wild type) of the ability to restore XRCC2 deficiency. The XRCC2 gene experiences an early translational stop signal (p.Cys217*) as a result of this sequence alteration. Furthermore, some hereditary cohorts of breast and/or ovarian cancer, including those with early-onset BC and male BC, have shown p.C217* [19, 21].

Single nucleotide change c.582G>T is classified as likely pathogenic in the ClinVar database and is located in the coding region of exon 3. It is classified as synonymous mutations, and no change is made in its amino acid sequence. The frequency of this type of mutation in the population is reported to be very low so that 1 person out of every 100,000 people has this mutation.

At position codon 61 within the XRCC2 protein, a mutation occurs where the amino acid leucine is replaced by isoleucine due to a sequence change (c.181C>A), resulting in a substitution of neutral and non-polar amino acids. This genetic variation, identified as rs569810249 in population databases such as gnomAD, is present in a small percentage (0.02%). Instances of this specific missense alteration have been documented in individuals with BC and stomach cancer [19, 22]. According to experimental research, the missense mutation has little effect on the XRCC2 function [23]. Since isoleucine and leucine have comparable qualities, this is seen as a cautious replacement of amino acids. XRCC2 Leu61Ile is not found in a recognized functional domain and is found at a location that is conserved across species. There is inconsistency in in silico investigations about the potential impact of this variation on the structure and function of proteins.

Within the 5' untranslated region (5' UTR) of the XRCC2 gene is where the c.-1G>A variation is located. G to A mutation occurs one nucleotide upstream of the first translated codon in this variation. This alteration was detected in 1/1308 early-onset BC patients and 0/1120 controls [19]. Based on nucleotide sequence alignment, the vertebrate species that are currently known to exist have a highly conserved nucleotide location. The therapeutic relevance of this modification is yet unknown because the supporting information is currently limited.

Considering the regenerative role of this gene, it is expected that its expression level will decrease during the process of tumorigenesis and have a tumor-suppressive nature; But this study showed that this gene is overexpressed in BC, and it can be considered an oncogenic nature. Previous studies prove this claim; These studies showed that XRCC is overexpressed in glioblastoma [24], colorectal [25], and BCs [26]. In contrast to the present study, it has been reported that lymph node (LN) metastatic BC tissue had downregulated XRCC2 [27]. The reason for this upregulated can be the effects of transcription factors affecting this gene or downstream signaling pathways.

The present study findings are not consistent with some other studies; It was shown in the study of Kluźniak *et al* [14]. In 52 out of 54 cases of cervical cancer (CC), it was discovered that the XRCC2 gene had hypermethylation in the promoter region. Promoter hypermethylation decreases gene expression, hence impairing the tumor-suppressing function of XRCC2 in HRal repair. Elevated levels of EZH2 result in the epigenetic suppression of RAD51 paralogs, such as XRCC2, hence diminishing HR repair [14, 28]. Also, Bashir *et al* [27] showed in their study that the decrease in the expression of this gene is related to the metastasis to the LNs, and significantly, the expression of this gene is decreased in cancer samples compared to healthy ones.

Epigenetic modification of the XRCC2 gene promoter has been detected in certain malignancies, such as Paulíková *et al* [29] established a strong correlation between promoter hypermethylation in the XRCC2 gene and the occurrence of severe grade III-IV toxicity in individuals with CC. This discovery holds potential for predicting late damage in patients who have undergone radiation. The increase in cancer risk is attributed to two well-known epigenetic factors resulting from XRCC2 loss. An example of this is the methylation of the XRCC2 gene promoter region, which leads to the epigenetic repression of XRCC2 due to the increased production of the EZH2 protein. The decrease in HR repair and the suppression of RAD51 paralogues, such as XRCC2, are accompanied by an upregulation of EZH2 expression. Elevated levels of EZH2 protein, ranging from 40% to 75%, have been observed in BC. Additionally, there is a roughly 7.5-fold rise in EZH2 mRNA expression [30].

5. Conclusion

As a conclusion our research revealed that there is a relation between XRCC2 and BC etiology. Based on the obtained results and previous studies, it can be claimed that this gene can be classified as an oncogene. Also, a significant relationship between age, disease grade, and type of BC with increased gene expression was reported. Considering the repairing function of this gene, it can be expected that its structural investigation by targeted NGS and expression evaluation can be used as a potential biomarker in BC.

Conflict of Interests

There are no disagreements between the author and any stage of the essay production.

Consent for publications

The author reviewed and gave their approval to the published version of the work.

Ethics approval and consent to participate
This study was conducted following the Declaration of HelsinkiParticipants were given informed consent, and the study was accepted by the Local Ethics Committee. Approval number: 05.01.2020\17.

Informed Consent
The Helsinki Declaration was followed in the conduct of this investigation. The Local Ethics Committee approved the study and participants provided informed permission.

Availability of data and material
The corresponding author can provide the study's data upon reasonable request.

Authors' contributions
The Authors contributed to this research work equally.

Funding
Non.

Acknowledgments
Our sincere gratitude goes out to everyone who contributed their time, effort, and expertise to make this study a success.

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