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ORIGINAL RESEARCH

Evaluation of *XRCC4* and *VEGF* Gene Variants in Relation to Breast Cancer Risk in a Jordanian Cohort

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Purpose: Breast cancer develops through complex interactions of genetic and environmental influences. *XRCC4* is a critical DNA repair gene that maintains genomic stability and is implicated in carcinogenesis, while *VEGF* is a key regulator of angiogenesis that plays an essential role in tumor progression and recurrence. The present study sought to evaluate the contribution of *XRCC4* (rs28360071) and *VEGF* (rs35569394) polymorphisms to breast cancer susceptibility in a Jordanian Arab cohort.

Patients and Methods: This case-control study included 300 breast cancer patients and 300 healthy individuals. Peripheral blood samples were collected for genomic DNA extraction. *XRCC4* (rs28360071) and *VEGF* (rs35569394) polymorphisms were genotyped using direct PCR, and their associations with breast cancer risk were analyzed using appropriate statistical methods.

Results: The *XRCC4* (rs28360071) polymorphism exhibited significant differences in allelic and genotypic distributions between breast cancer cases and control individuals in both unadjusted ($p = 0.01$) and adjusted analyses ($p = 0.005$), with the DD genotype associated with reduced breast cancer risk.

Conclusion: The findings suggest that *XRCC4* (rs28360071) is associated with breast cancer susceptibility among Jordanian Arab women, whereas *VEGF* (rs35569394) does not appear to influence risk. These results indicate a potential role for *XRCC4* variation in breast cancer susceptibility in this population; however, this finding should be cautiously interpreted, as further research is required to confirm the association.

Keywords: breast cancer, polymorphism, VEGF, XRCC4

Introduction

Breast cancer represents the most prevalent cause of cancer-related deaths in women across the world, it develops through a complex interaction of endo- and exogenous influences.[1–3] Whereas most cases of breast cancer occur sporadically, about 10–15% are hereditary arising from genetic mutations that can be transmitted from parent to offspring.[4] Familial cancers, as a distinct category accounts for 15–20% of cases in which a hereditary component is suspected despite the absence of a clearly identified causative mutation, these cancers likely arise from a combination of inherited susceptibility and shared environmental or socioeconomic factors.[5]

DNA repair pathways are critical for preserving genomic stability and integrity by rectifying DNA damage that could otherwise promote carcinogenesis.[6] Genetic alterations in DNA repair and damage signaling genes have been frequently identified in human malignancies; therefore, it's reasonable to hypothesize specific genetic variants in DNA repair genes including X-ray cross complementing group 4 (*XRCC4*) and may contribute to breast cancer pathogenesis.[7,8] The *XRCC4* gene, which plays a significant role in the non-homologous end-joining (NHEJ) repair pathway, has been demonstrated to restore DNA double strand break (DSB) repair and facilitate V(D)J recombination of transiently introduced substrates in the XR-1 CHO cell line.[9]

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XRCC4 is essential for the accurate repair of blunt DNA double strand breaks (DSBs) in mammalian fibroblasts.[10] It has been documented that there is a notable correlation between elevated cancer susceptibility and the synergistic effect of single nucleotide polymorphisms (SNPs) in NHEJ genes.[11] The widely recognized breast cancer susceptibility gene *BRCA1* may contribute to cancer risk through modulation of cellular NHEJ capability.[12] Additionally, it has been observed that in hereditary cases of non-*BRCA1/2* breast cancer, *XRCC4* may influence the age at diagnosis and the risk of the disease.[13] Several documented SNPs in the *XRCC4* gene have been reported to correlate with incidences of gastric, bladder and breast cancer.[14–16] Among the less well-characterized polymorphisms in *XRCC4*, rs28360071, located in intron 3, has been investigated in relation to susceptibility to various malignancies, including colorectal, prostate, and oral cancers;[17–19] however, its association with breast cancer remains unclear.

Angiogenesis is a feature that characterizes cancer and is controlled by a variety of growth factors,[20] including platelet-derived growth factors, transforming growth factors and angiopoietins, with a crucial contribution from vascular endothelial growth factors (VEGF).[21–23] VEGF functions as one of the most potent mitogens for endothelial cells that play an essential role in both normal physiological and tumor angiogenesis.[24–26] It promotes the permeability of tumor vessels and stimulates endothelial cell proliferation, migration, differentiation and capillary formation, while also exhibiting proinflammatory effects.[27–29]

It is involved in tumor-related pathological angiogenesis, which is a crucial step in cancer development essential for primary tumor growth, invasiveness, and metastasis.[30] Overexpression of VEGF has been observed in various tumor tissues.[31–33] Breast cancer is one of the most well-known malignancies involving lymphangiogenesis, the process by which blood and lymphatic vessels are attracted to an expanding tumor.[34] Studies conducted in vitro and in vivo demonstrate that elevated VEGF expression correlates with tumor growth and metastasis. Furthermore, blocking VEGF signaling suppresses tumor-induced angiogenesis and inhibits tumor growth.[35]

Polymorphic variations located in the promoter 5′-UTR and 3′-UTR of the *VEGF* gene have been documented to influence translation efficiency, circulating plasma levels and its expression in tumor tissues.[36,37] *VEGF* polymorphisms that affect VEGF expression in normal cells have been documented to influence tumorigenesis, tumor progression and the effectiveness of anti-VEGF agents.[30,38–40] Among these, the rs35569394 polymorphism, located at position −2549 in the promoter region of *VEGF*, has been associated with altered transcriptional activity.[41] However, limited studies have investigated the association between rs35569394 and breast cancer susceptibility across different populations, with inconsistent findings.[42,43]

Further, the development of breast cancer involves the cooperation of genetic, environmental, and biological factors, focusing attention on the importance of identifying polymorphisms that may influence disease risk. *XRCC4*, a key component of the non-homologous end-joining DNA repair pathway, and *VEGF*, a central regulator of angiogenesis, have been implicated in cancer development. However, limited and inconsistent data are available regarding the association of specific polymorphisms, including rs28360071 and rs35569394, with breast cancer risk. Moreover, evidence from Middle Eastern populations, including Jordanian Arabs, remains scarce. Given that factors such as consanguinity and regional admixture may influence genetic architecture, allele frequencies and gene-disease associations, this study aims to investigate the potential association of *XRCC4* and *VEGF* polymorphisms with breast cancer susceptibility in a Jordanian Arab population.

Materials and Methods

Study Participants

This study included 600 participants (300 breast cancer patients and 300 unrelated healthy female controls), the patients were recruited based on specific criteria from the chemotherapy clinics of King Abdullah University Hospital (KAUH) and King Hussein Medical Center. The control group was recruited from the general population and matched to the patients' age, whereas both patient and control participants shared a common Jordanian ethnic background. Ethical approval for this study was obtained from the Institutional Review Board (IRB) of Jordan University of Science and Technology and KAUH (Approval No: 9/143/2021) in accordance with the Declaration of Helsinki. Prior to participation, all subjects signed written informed consent forms.

Patients were enrolled in the study if they met predefined inclusion criteria, which required a histopathologically confirmed diagnosis of breast cancer, no evidence of HIV, HBV, or HCV infection and availability of clinical records in the KAUH registry. Patients were excluded if they refused to give written informed consent, had undergone a blood transfusion during surgery or lacked sufficient clinical data.

The control participants were included if there was no documented history of breast cancer or other malignancies. Participants were recruited from the same ethnic background to minimize the potential for population stratification bias. Participants were excluded from the control group if they reported a history of cancer, a first-degree family history of breast cancer, chronic infectious conditions, or recent blood transfusion. Those who declined to provide informed consent or biological samples were excluded from the study.

DNA Extraction and Quality Assessment

Genomic DNA was extracted from frozen whole blood samples using the Puregene® Blood Core Kit A (Qiagen, USA) according to the procedure described in the user guide. The quality and concentration of DNA were determined using a NanoDrop spectrophotometer. However, the DNA samples accepted must have A260/A280 absorption ratio values between 1.8 and 2.0, and the DNA samples with insufficient purity were re-extracted to ensure suitability for downstream analyses, and the DNA integrity was evaluated using 1% agarose gel electrophoresis to verify the absence of degradation. Quality control of genotyping was ensured by re-genotyping randomly selected samples. Samples with unclear or ambiguous results were re-genotyped to confirm accurate genotype calls. Any samples with missing data were excluded from the analysis to maintain dataset consistency.

Genotyping of VEGF rs35569394 Polymorphism

According to the *VEGF* rs35569394 polymorphism, polymerase chain reaction (PCR) was utilized to amplify the specific target sequence with two oligonucleotide primers (5′-GCTGAGAGTGGGGCTGACTAGGTA-3′ and 5′-GTTTCTGACCTG GCTATTTCCAGG-3′). The thermal cycling conditions comprised an initial denaturation at 95 °C for 6 min, followed by 35 cycles of denaturation (95 °C, 30sec), annealing (59 °C, 30sec) and extension (72 °C, 30sec), with a final extension at 72 °C for 10 min. The products were electrophoresed on a 3% agarose gel. The presence of the D allele produced a 211 bp fragment, while the I allele produced a 229 bp fragment.

Genotyping of XRCC4 rs28360071 Polymorphism

According to the *XRCC4* rs28360071 polymorphism, polymerase chain reaction (PCR) was utilized to amplify the specific target sequence with two oligonucleotide primers (5′-TCCTGTTACCATTTCAGTGTTAT-3′ and 5′-CACCTGTGTTCAATTCCAGCTT-3′). The thermal cycling conditions comprised an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation (95 °C, 30s), annealing (55 °C, 30s) and extension (72 °C, 30s), with a final extension at 72 °C for 10 min. The products were electrophoresed on a 3% agarose gel. The presence of the D allele produced a 109 bp fragment, while the I allele produced a 139 bp fragment.

Statistical Analysis

The statistical evaluation of the data was carried out using the SNPStats web tool (https://www.snpstats.net/start.htm) to test Hardy-Weinberg equilibrium, determine allelic and genotypic distributions, and evaluate different inheritance dominant, recessive and overdominant models. The strength of association was quantified using odds ratio (OR) with corresponding 95% confidence intervals (CI) and statistical significance was defined at the $p < 0.05$ threshold. The more frequent allele in the control group was defined as the reference allele, while the less frequent allele was considered the effect allele. Multivariable logistic regression analysis was used to evaluate the association between genetic polymorphisms under a codominant model and breast cancer risk after adjustment for relevant confounding variables. Associations between genotypic categories and phenotypic traits were assessed using Pearson's chi-square test and one-way ANOVA, performed in SPSS software, version 26.0 (SPSS Inc., Chicago, IL). Survival analysis was performed using the Kaplan–Meier method in GraphPad Prism (version 10.6.1), and differences between groups were assessed using the Log rank test. Time-to-event was defined as the period from breast cancer diagnosis to death or last follow-up. An adjustment for

multiple comparisons was implemented by calculating the relevant number of polymorphisms based on a method outlined in a prior study.[44] The level of statistical significance was adjusted according to the Bonferroni method (α/n), where α was set at 0.05 and n denoted the total number of performed tests.[45]

Results

Baseline Characteristics of Patients and Controls

All included participants were of Jordanian Arab ethnicity, with baseline demographic characteristics comparable across patient and control groups. No significant differences were observed between patients and controls in mean age (52.32 ± 11.39 vs. 52.35 ± 11.24 years, p = 0.97), BMI (28.86 ± 5.98 vs. 28.36 ± 5.92, p = 0.30), smoking status (14.2% vs. 16.6%, p = 0.44), or age at menarche (13.73 ± 1.65 vs. 13.82 ± 1.33, p = 0.52). Patients had a significantly higher frequency of family history of cancer than controls (54.7% vs. 36.0%, p < 0.001).

Assessment of Hardy–Weinberg Equilibrium (HWE)

Table 1 presents the Hardy-Weinberg Equilibrium (HWE) assessment and corresponding minor allele frequencies (MAF) for two polymorphisms, rs35569394 and rs28360071, in both breast cancer patients and controls. For the *VEGF* (rs35569394) polymorphism, the minor allele (I) frequency was 42% in cases and 43% in controls, with HWE p-values of 1 and 0.72, respectively, indicating no significant deviation from equilibrium. For the *XRCC4* (rs28360071) polymorphism, the minor allele (D) frequency was 42% in cases and 49% in controls, with HWE p-values of 0.72 and 0.49, respectively.

Genetic Association of Polymorphisms with Breast Cancer Susceptibility

Table 2 presents the genetic association of studied polymorphisms between breast cancer patients and healthy controls, with both unadjusted and adjusted analyses controlling for potential confounding variables, including age, BMI, smoking status, age at menarche and family history. The distribution of *VEGF* alleles and genotypes did not differ significantly between patients and controls, suggesting no detectable association with breast cancer in the cohort studied. However, *XRCC4* demonstrated significant differences in allelic and genotypic distributions in both unadjusted (OR = 0.55, 95% CI: 0.34–0.86, p = 0.010) and adjusted analyses (OR = 0.48, 95% CI: 0.28–0.80, p = 0.005), with the homozygous DD genotype presenting a reduced risk of breast cancer.

Table 1 Hardy–Weinberg Equilibrium Analysis and Minor Allele Frequency Distribution in Cases and Controls

Gene/Polymorphism	Allele/Genotype	Allelic/Genotypic Frequencies (%)			
		Cases (n=300)	HWE p-value	Controls (n=300)	HWE p-value
VEGF (rs35569394)	I^{MA}	250 (42%)^{MAF}	I	256 (43%)^{MAF}	0.72
	D	350 (58%)		344 (57%)	
	II	52 (17%)		56 (19%)	
	ID	146 (49%)		144 (48%)	
	DD	102 (34%)		100 (33%)	
XRCC4 (rs28360071)	I	349 (58%)	0.72	304 (51%)	0.49
	D^{MA}	251 (42%)^{MAF}		296 (49%)^{MAF}	
	II	103 (34%)		80 (27%)	
	ID	143 (48%)		144 (48%)	
	DD	54 (18%)		76 (25%)	

Abbreviations: MA, Minor Allele; MAF, Minor Allele Frequency; I, Insertion; D, Deletion.

Table 2 Genetic Association of Polymorphisms in Unadjusted and Adjusted Analyses of Breast Cancer Cases and Controls

Polymorphism	Allelic/Genotypic Frequencies in BC Patients and Control						
	Allele/Genotype	Cases (N = 300)	Control (N = 300)	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
VEGF (rs35569394)	D I	350 (58%) 250 (42%)	344 (57%) 256 (43%)	1 0.95 (0.76–1.21)	0.725	–	–
	DD ID II	102 (34%) 146 (49%) 52 (17%)	100 (33%) 144 (48%) 56 (19%)	1 0.99 (0.69–1.41) 0.91 (0.57–1.43)	0.973 0.693	1 1.01 (0.67–1.52) 0.99 (0.59–1.69)	0.945 0.997
XRCC4 (rs28360071)	I D	349 (58%) 251 (42%)	304 (51%) 296 (49%)	1 0.73 (0.58–0.92)	0.009	–	–
	II ID DD	103 (34%) 143 (48%) 54 (18%)	80 (27%) 144 (48%) 76 (25%)	1 0.77 (0.53–1.12) 0.55 (0.34–0.86)	0.171 0.0101	1 0.70 (0.46–1.08) 0.48 (0.28–0.80)	0.108 0.005

Notes: P-values < 0.0125 (0.05/4 = 0.0125 after applying multiple comparisons) are considered significant. The control group was used as the reference category for all case-control comparisons, while the wild-type genotype was used as the reference category for genetic models. Adjusted analyses included age, BMI, smoking status, age at menarche, and family history of cancer. The same covariate set was applied to all adjusted models.
Abbreviations: OR, odds ratio; CI, confidence interval.

Genetic Association Under Different Genetic Models

Table 3 examines the genetic models and distribution of specific gene polymorphisms (VEGF rs35569394 and XRCC4 rs28360071) in relation to case-control status. For the VEGF polymorphism, none of the genetic models (dominant, recessive and overdominant) exhibited significant associations with breast cancer ($p > 0.008$), suggesting no correlation between VEGF rs35569394 and breast cancer risk in this study. Comparably, the XRCC4 polymorphism was not significantly associated with breast cancer risk under any genetic model after applying correction.

Table 3 The Genetic Models and Distribution of the Studied Gene Polymorphisms in Response to Case-Control

Polymorphism	Model	Genotype	Cases (%)	Controls (%)	OR (95% CI)	p- value
VEGF (rs35569394)	Dominant	D/D I/D-I/I	102 (34%) 198 (66%)	100 (33.3%) 200 (66.7%)	1.00 0.97 (0.69–1.36)	0.86
	Recessive	D/D-I/D I/I	248 (82.7%) 52 (17.3%)	244 (81.3%) 56 (18.7%)	1.00 0.91 (0.60–1.39)	0.67
	Overdominant	D/D-I/I I/D	154 (51.3%) 146 (48.7%)	156 (52%) 144 (48%)	1.00 1.03 (0.75–1.41)	0.87
XRCC4 (rs28360071)	Dominant	I/I I/D-D/D	103 (34.3%) 197 (65.7%)	80 (26.7%) 220 (73.3%)	1.00 0.70 (0.49–0.99)	0.041
	Recessive	I/I–I/D D/D	246 (82%) 54 (18%)	224 (74.7%) 76 (25.3%)	1.00 0.65 (0.44–0.96)	0.029
	Overdominant	I/I-D/D I/D	157 (52.3%) 143 (47.7%)	156 (52%) 144 (48%)	1.00 0.99 (0.72–1.36)	0.93

Note: P-values < 0.0083 (0.05/6 = 0.0083 after applying multiple comparisons) are considered significant.
Abbreviations: OR, odds ratio; CI, confidence interval.

Clinical Implications of VEGF and XRCC4 Polymorphisms in Breast Cancer Outcomes

Table 4 presents the phenotype-genotype associations between *VEGF* rs35569394 and *XRCC4* rs28360071 polymorphisms and various clinical characteristics of breast cancer patients. After applying Bonferroni correction for multiple testing, the *VEGF* polymorphism showed a significant association with allergy ($p < 0.0001$), while *XRCC4* was significantly associated with family history of cancer ($p < 0.0001$). These findings represent corrected statistical associations derived from multiple phenotype–genotype comparisons and should therefore be interpreted with caution in this regard.

Assessment of Survival Outcomes in Breast Cancer Patients

Kaplan–Meier survival analysis, followed by the Log rank test, was conducted to evaluate overall survival. The median follow-up was 3 years, during which five mortality events (deaths) were recorded. Censoring was applied to patients alive at last follow-up. The analysis included all eligible breast cancer patients stratified according to *VEGF* (rs35569394) and *XRCC4* (rs28360071) genotypes. The analysis demonstrated no statistically significant difference in survival time

Table 4 The Phenotype-Genotype Association Between rs35569394 and rs28360071 Genotypes and the Clinical Outcomes of Breast Cancer

Clinical Outcome	VEGF (rs35569394) p- value	XRCC4 (rs28360071) p- value
Age	0.7273	0.8406
Age at BC Diagnosis	0.7835	0.6889
Stage of BC	0.7522	0.7815
Age at First Pregnancy	0.2005	0.5277
Body Mass Index	0.8541	0.0694
Age of Menarche	0.2774	0.6395
Age of Menopause	0.4329	0.6928
Breastfeeding Status	0.4278	0.0043
Family History of Cancer	0.1052	<0.0001
Other Types of Cancer	0.1949	0.0407
Polycystic ovary syndrome (PCOS)	0.2156	0.8459
Uterine Fibroid	0.1456	0.1905
Benign Breast Tumor	0.6762	0.8114
Estrogen Receptor	0.3221	0.1053
Progesterone Receptor	0.7639	0.3683
Human Epidermal Growth Factor Receptor 2 (HER2)	0.4394	0.7014
Axillary Lymph Node Metastasis	0.4278	0.5072
Lymph vascular Invasion	0.6285	0.8169
Distant Metastasis	0.1997	0.2225
Allergy	<0.0001	0.6003
Smoking	0.4593	0.0694

Notes: Statistical analysis was performed using the ANOVA test for continuous variables and the chi-square test for categorical variables. Sample size varied slightly due to missing data; all available cases were included in each analysis. P-values < 0.0012 (0.05/42 = 0.0012 after applying multiple comparisons) are considered significant.

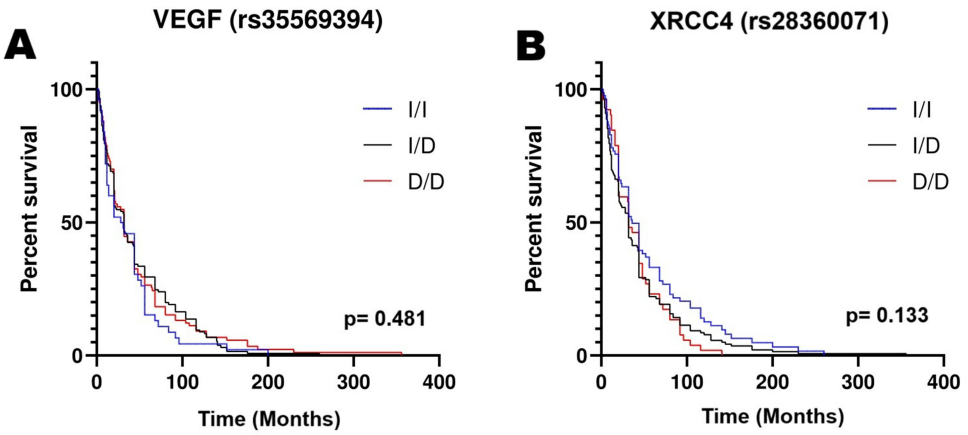

Figure 1 Kaplan–Meier survival curves showing the association between genotype and overall survival (OS) in breast cancer patients for (**A**) *VEGF* rs35569394 and (**B**) *XRCC4* rs28360071 polymorphisms. Survival differences between genotype groups were assessed using the Log rank test. The median follow-up was 3 years, and five death events were recorded.

associated with *VEGF* or *XRCC4* polymorphisms in breast cancer patients (p = 0.481 and p = 0.133, respectively), as illustrated in Figure 1A and B.

Discussion

Although substantial progress has been made in the diagnosis and treatment of breast cancer, current therapeutic strategies have not fully realized the expected reductions in morbidity and mortality; however, identifying critical molecular targets and elucidating the molecular mechanisms underlying carcinogenesis are both crucial for the prevention and development of effective breast cancer therapies.

The human *VEGF* gene is known to be highly polymorphic with essential variability in its expression levels.[38] Plasma VEGF levels are strong predictors of tumor progression and overall survival in breast cancer patients,[46,47] but its overexpression has been shown to support the maintenance of vascular networks in multiple tumor types, including breast cancer.[48] Recent evidence indicates that multiple mutations in the *VEGF* gene are associated with breast cancer risk and susceptibility.[49,50]

Our study identified the 18-bp indel (rs35569394) polymorphism in the *VEGF* promoter and found no association between rs35569394 and breast cancer susceptibility. In the Iranian population, Rezaei et al study did identify the (rs35569394) polymorphism but with no significant link to breast cancer susceptibility;[42] contrarily, studies in North India reported that the II genotype and I allele of the rs35569394 polymorphism were significantly linked to increased breast cancer susceptibility.[43,51] In Chinese women also, the DD genotype of rs35569394 was found to significantly raise breast cancer susceptibility.[52] The lack of association observed for *VEGF* rs35569394 in this study contributes to the growing body of evidence suggesting that the effect of this polymorphism may vary across ethnic populations. This finding supports the hypothesis that genetic susceptibility to breast cancer may be influenced by population-specific factors, which include genetic background and environmental exposures, underscoring the importance of considering these factors in risk assessment.

DNA repair protects an organism from carcinogenesis; hence, DNA repair gene polymorphisms are viewed as breast cancer risk factors.[53] The *XRCC4* gene plays a pivotal role as an indicator of genomic stability and has been reported as one of the most crucial contributors to human carcinogenesis.[8] Moreover, genetic polymorphism of DNA repair genes is assumed to modulate the capacity of DNA repair regarding breast cancer.[53,54] Although the significance of *XRCC4* has been identified, there is inadequate research that has analyzed the relationship regarding the *XRCC4* polymorphism and risk of breast cancer.[16]

Several functional *XRCC4* gene polymorphisms have been implicated as risk factors for breast cancer with different variants consistently observed to be associated with the disease across independent studies.[13,55] Our study identified the 30-bp indel (rs28360071) polymorphism in *XRCC4* and found an association between this variant and breast cancer susceptibility. We observed that the DD genotype and D allele of the rs28360071 polymorphism were significantly associated with reduced breast cancer susceptibility in the studied Jordanian Arab population; this association was

consistent across unadjusted and adjusted analyses for confounding, with the DD genotype appearing to have a protective effect. In contrast, studies in the Iranian population reported no significant association between the rs28360071 polymorphism and breast cancer susceptibility.[56]

The differences between our findings and those reported in other populations may reflect factors beyond ethnic variation, including differences in genetic architecture such as allele frequencies and linkage disequilibrium patterns across populations, which can influence variant effects on disease susceptibility.[57,58] Gene-environment interactions may also contribute, as lifestyle, reproductive, and environmental exposures differ across populations and may modify genetic effects.[59] In addition, variation in study design, sample size, and adjustment for confounders may explain inconsistencies between studies.[60,61] Overall, these factors suggest that the effects of *VEGF* and *XRCC4* polymorphisms on breast cancer susceptibility are likely population-specific and context-dependent rather than universally applicable.

In addition to the primary genetic association results, the phenotype-genotype associations observed in this study should be interpreted cautiously, as these analyses were exploratory and involved multiple comparisons. Although some associations remained statistically significant after Bonferroni correction, the possibility that certain findings represent chance observations cannot be excluded. Thus, the findings cannot be used as definitive proof of biological connections but only as possible markers that need confirmation; larger independent studies are required to confirm the robustness and reproducibility of these associations before any firm conclusions can be drawn.

The Kaplan-Meier analysis with Log rank testing showed no significant differences in overall survival according to *VEGF* rs35569394 or *XRCC4* rs28360071 genotypes. As a limited number of events, multivariable Cox regression could not be reliably performed, and hence only unadjusted survival analyses were conducted. These results should be interpreted cautiously as exploratory findings that do not account for potential confounding and require validation in larger studies.

Despite the valuable insights gained from the present study, several limitations should be acknowledged. A formal sample size calculation and power analysis were not performed, as the study was based on available recruitment. This may limit statistical power, particularly for secondary and survival analyses and the findings should therefore be interpreted as exploratory. In addition, the relatively modest sample size may reduce the ability to detect weaker associations or gene-gene interactions; also, the study population was restricted to Jordanian Arab women potentially limiting the generalizability of the findings to other ethnic groups. Only selected polymorphisms in *XRCC4* and *VEGF* were investigated, whereas additional variants in these and other DNA repair or angiogenesis-related genes may also influence breast cancer susceptibility. Moreover, the technique that was used for genotyping was a direct PCR method. Although this would be more efficient, it would be limited when it comes to sensitivity and accuracy compared to high-throughput techniques. Prospective research should target broader, multi-center cohorts to verify these results and elucidate the contributions of both gene-gene and gene-environment interactions. Moreover, functional studies are warranted to elucidate the biological mechanisms by which these polymorphisms affect DNA repair capacity, angiogenesis and ultimately breast cancer risk; such efforts may contribute to improving understanding of the underlying molecular pathways.

Conclusion

The present study suggests that the DD genotype and D allele of the rs28360071 polymorphism in the *XRCC4* gene are associated with decreased breast cancer susceptibility in the Jordanian Arab population, which may indicate a protective effect. In contrast, *VEGF* polymorphisms were not associated with breast cancer risk. These findings highlight a potential population-specific role of *XRCC4* genetic variation in breast cancer susceptibility. Further studies with larger sample sizes are warranted to validate these findings and to investigate the underlying biological mechanisms.

Data Sharing Statement

The primary data used to support the findings of this study are available from the corresponding author upon request.

Ethics Approval and Informed Consent

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board committee at The Jordan University of Science and Technology (No: 9/143/2021). Written informed consent was obtained from all participants before they participated in the study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest.

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