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Association of FGFR2 Gene Polymorphisms (rs2981582 and rs1219648) with Breast Cancer Susceptibility in Iranian Women: A Case-Control Study with Haplotype and Expression Analysis

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ABSTRACT

Background: Breast cancer is the most common malignancy among women in Iran, characterized by a relatively early age of onset and a rising incidence rate. The single-nucleotide polymorphisms rs2981582 and rs1219648, located in intron 2 of the *FGFR2* gene, have been linked to breast cancer susceptibility in genome-wide association studies (GWAS). Nevertheless, their significance in the Iranian population has not been extensively investigated.

This study investigates the association of *FGFR2* polymorphisms (rs2981582 and rs1219648) with breast cancer risk in Iranian women, alongside haplotype interactions and gene expression profiling.

Methods: A case-control study was conducted with 160 participants (80 cases and 80 age-matched controls). *FGFR2* SNPs were genotyped with PCR-RFLP. Chi-square tests were used to analyze associations of haplotypes. *FGFR2* expression was evaluated in breast cancer subtypes using GEO (GDS2635, GDS3853) and Expression Atlas datasets. Statistical analyses were carried out using SPSS version 22.0 (IBM Corp., Armonk, NY, USA), with statistical significance defined as $P<0.05$. Hardy–Weinberg equilibrium (HWE) was verified for both SNPs in the control group ($P>0.05$).

Results: The TT genotype of rs2981582 was significantly associated with increased breast cancer risk ($P=0.00$; OR=3.566). No independent association was found for rs1219648 ($P>0.05$). Haplotypes AC and AT were significantly associated with elevated risk ($P=0.004$ and $P=0.001$, respectively). *FGFR2* expression was upregulated in lobular carcinoma and downregulated in ductal carcinoma compared to healthy controls ($P<0.05$).

Conclusion: The rs2981582 TT genotype and specific haplotypes (AC, AT) are associated with increased breast cancer risk in Iranian women, supporting *FGFR2* as a potential biomarker for early detection and personalized risk assessment in this population.

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INTRODUCTION

Breast cancer continues to be the most commonly diagnosed malignancy and the primary cause of cancer-related mortality among women globally, with more than 2.3 million new cases and nearly 685,000 deaths reported annually according to GLOBOCAN 2020.

Despite improvements in screening and treatment worldwide, the burden of this disease is increasing rapidly in low- and middle-income countries, including Iran, where it represents the most prevalent cancer among women and shows a concerning pattern of earlier onset compared to Western populations (1).

In recent years, growing attention has been directed toward genetic susceptibility factors associated with the risk of Breast cancer, particularly single-nucleotide polymorphisms (SNPs). Intronic variants in the *FGFR2* gene have been identified as significant contributors in several population-based genome-wide association studies (GWAS) (2, 3). The *FGFR2* gene encodes a receptor tyrosine kinase involved in regulating cell proliferation, differentiation, and angiogenesis, and disruption of these processes can contribute to tumor development. Notably, the SNPs rs2981582 and rs1219648, located within intron 2 of the *FGFR2* gene, have been associated with alterations in transcription factor binding and gene expression, thereby increasing susceptibility to breast cancer (2).

Despite a vast number of association studies being conducted around the world, the Iranian population has not been notably represented in genetic epidemiology studies. Initial studies from regional locations, such as Northern and Azeri Iranian subpopulation studies, provided inconsistent or insufficient evidence for a role of *FGFR2* SNPs in susceptibility to breast cancer (4, 5).

Furthermore, there is a paucity of data integrating SNP analysis with gene expression profiles to explore the functional consequences of these variants.

This study aims to investigate the role of relevant *FGFR2* polymorphisms (rs2981582 and rs1219648) in breast cancer risk in an Iranian female population. This study also analyses the expression patterns of *FGFR2* in different histological subtypes of breast cancers by way of bioinformatics datasets. Our findings can provide insight into the understanding of genetic risk factors for Iranian women and can help develop population-specific markers for early detection.

MATERIALS AND METHODS

Study Design and Participants

A case-control study was conducted with 160 Iranian women (80 breast cancer cases and 80 age-matched controls) recruited from hospitals in Markazi province. The cases were histologically confirmed breast cancer patients, and the controls were asymptomatic women with no personal or family history of cancer. Inclusion criteria for cases included a confirmed diagnosis of primary breast cancer, while controls were excluded if they had any history of malignancy or family history of breast cancer. Ethical approval was obtained from the Ethics Committee of Arak University (IR.ARAKMU.REC.1395.288), and written informed consent was provided by all participants.

Sample Collection and DNA Extraction

Peripheral blood samples (5 mL) were obtained and collected into EDTA-containing tubes. Genomic DNA was isolated using the YTA Genomic DNA Extraction Mini Kit (Yekta Tajhiz Azma, Iran) in accordance with the manufacturer's instructions. DNA concentration and purity were evaluated using UV spectrophotometry (Nanodrop), and its integrity was confirmed by electrophoresis on a 2% agarose gel.

Genotyping of *FGFR2* SNPs

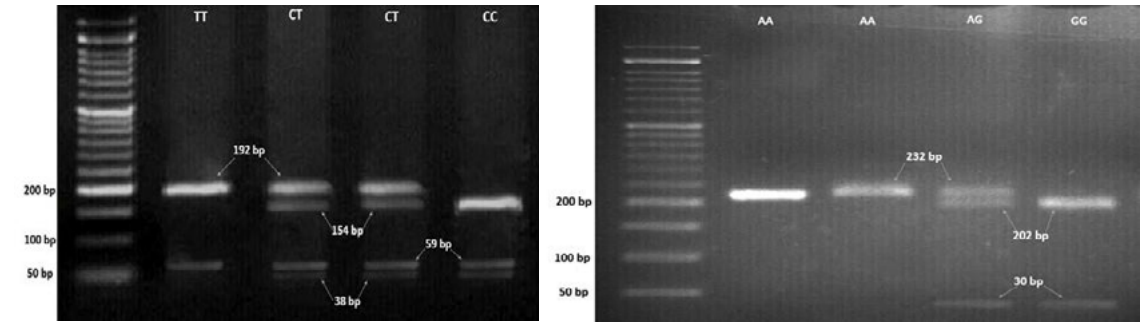
SNPs rs2981582 (T>C) and rs1219648 (A>G) in intron 2 of *FGFR2* were genotyped using Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP). PCR amplification was performed in a 25 µL reaction volume containing genomic DNA, primers, dNTPs, buffer, $MgCl_2$, and Taq polymerase. Thermal cycling was performed with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles consisting of 95°C for 30 seconds, 58°C (rs2981582) or 60°C (rs1219648) for 30 seconds, and 72°C for 40 seconds, with a final extension at 72°C for 7 minutes. Primers (Table 1) were validated using the NCBI Primer-BLAST. PCR products were digested with *AciI* (rs2981582) and *LguI* (rs1219648) at 37°C for 16 hours, then resolved on a 2% agarose gel and visualized under UV illumination (Figure 1). Genotype distributions in the control group were assessed for Hardy–Weinberg equilibrium using a chi-square test.

Gene Expression Analysis

Public bioinformatics platforms yielded gene expression data that allows for *FGFR2* expression to

Table 1. Sequence of primers used in the amplification of the FGFR2 gene polymorphisms

Gene	SNP	Primer Seq	Product size (bp)
FGFR2	rs1219648	F: 5'-ACGCCTATTTTACTTGACACGC R: 5'- GCTGGACAGGTCATTGTGGTG	232
FGFR2	rs2981582	F: 5'- CCCTTTGGAGACAACGTGAGC R: 5'- GCACGAGATGTGTTCCCAGAG	251



(A) rs2981582: TT genotype yields an uncut 192 and 59 bp band; TC genotype shows 192, 154, 59, and 38 bp bands; CC genotype shows 154, 59 and 38 bp bands.

(B) rs1219648: AA genotype remains uncut at 232 bp; AG genotype shows 232, 202, and 30 bp bands; GG genotype displays 202 and 30 bp bands.

Fig 1. Agarose gel electrophoresis patterns of sample RFLP-PCR products for SNPs in the FGFR2 gene

be characterized across different histological subtypes of breast cancer.

This was accomplished by obtaining GEO Datasets (GDS2635 and GDS3853) through NCBI GEO, and also from the Expression Atlas (EBI) for profiling *FGFR2* expression in normal and tumor breast tissues.

Expression levels were compared in ductal and lobular subtypes and in healthy reference individuals.

Statistical Analysis

Genotype and allele distributions were compared using chi-square tests. Odds ratios (ORs) along with 95% confidence intervals (CIs) were calculated to evaluate the strength of associations. Multivariate logistic regression analysis was applied to control for potential confounding factors, including age, menopausal status, family history, smoking, and oral contraceptive use. Haplotype frequencies were estimated using Haploview. Power analysis performed with G*Power indicated that the sample size (n=160) achieved 80% power to detect an OR of 3.5 at α=0.05. Statistical analyses were conducted using SPSS version 22.0 (IBM Corp., Armonk, NY, USA), with P<0.05 considered statistically significant.

RESULTS

Demographic Characteristics of Study Participants

Demographic analysis (Table 2) showed that cases had a slightly higher mean age (49.8 ± 9.1 years) than controls (47.2 ± 8.7 years; P=0.07). Cases were more likely to be postmenopausal (75.0% vs. 60.0%, P=0.03), have a family history of breast cancer (33.8% vs. 13.8%, P=0.004), lower education levels (≤ high school: 82.5% vs. 56.3%, P=0.001), smoking history (18.8% vs. 7.5%, P=0.03), and oral contraceptive use (65.0% vs. 50.0%, P=0.05).

Genotypic and Allelic Frequencies

Genotype frequencies for rs2981582 and rs1219648 were in Hardy-Weinberg equilibrium in controls (P=0.13 and P=0.65, respectively). The TT genotype of rs2981582 was significantly more frequent in cases

Table 2. Investigating the relationship between clinical variables and the risk of breast cancer

Variable	Cases (n=80)	Controls (n=80)	P-value
Mean Age (years)	49.8 ± 9.1	47.2 ± 8.7	0.07
Postmenopausal (%)	60 (75.0%)	48 (60.0%)	0.03
Family History of BC (%)	27 (33.8%)	11 (13.8%)	0.004
Education Level ≤ High School (%)	66 (82.5%)	45 (56.3%)	0.001
Smoking (%)	15 (18.8%)	6 (7.5%)	0.03
Oral Contraceptive Use (%)	52 (65.0%)	40 (50.0%)	0.05

(52.5%) than controls (26.3%), with a strong association with breast cancer risk (P=0.001; OR=3.566, 95% CI: 1.519-8.005). After adjusting for confounders (age, menopausal status, family history, smoking, and oral contraceptive use), the association remained significant (adjusted OR=3.20, 95% CI: 1.40-7.80, P=0.002). No significant association was observed for rs1219648 genotypes (P>0.05). Allelic analysis showed that the T allele of rs2981582 was significantly associated with increased breast cancer risk, while the G allele of rs1219648 showed no significant difference (Table 3). Analysis of recessive and dominant models for rs2981582 confirmed a stronger association in the recessive (TT vs. TC+CC: OR=3.56, 95% CI: 1.58- 8, P=0.002) and Dominant (CC vs. TC + TT: OR=3.8, 95% CI: 1.92-7.54, P=0.00) models. Agarose gel electrophoresis patterns of PCR-RFLP products are shown in Figure 1.

In Figure 1, agarose gel electrophoresis patterns of sample RFLP-PCR products for SNPs in the *FGFR2* gene are shown.

Haplotype Analysis

Haplotype frequencies were estimated using Haploview software. Four haplotypes (AC, GC, AT, GT) were identified, with AC (frequency: 0.42 in cases vs. 0.28 in controls) and AT (frequency: 0.35 in cases vs. 0.20 in controls) showing significant associations with increased breast cancer risk (P=0.004 and P=0.001, respectively; Table 4). The overall haplotype distribution differed significantly between cases and controls (χ^2=21.914, df=3, P=0.00).

Table 3. Study of genotypic and allelic frequencies of the FGFR2 gene SNPs in patient and control groups

SNP	Genotype	Cases (n=80)	Controls (n=80)	OR (95%CI)	P-value	HWB
rs2981582	CC	18 (22.5%)	42 (52.5%)	-	-	0.13
	TC	35 (43.8%)	28 (35%)	2.91 (1.38-6.13)	**0.004**	
	TT	27 (33.7%)	10 (12.5%)	3.566 (1.519-8.005)	**0.00**1	
	TT vs	27 (22.5%)	10 (12.5)	3.56 (1.58- 8)	**0.002**	
	TC + CC	53 (77.5%)	70 (87.5%)			
	CC vs	18 (22.5%)	42 (52.5%)	3.8 (1.92-7.54)	**0.00**	
	TC + TT	62 (77.5%)	38 (47.5%)			
rs1219648	AA	33 (41.3%)	36 (45%)	-	-	0.65
	AG	36 (45%)	34 (42.5%)	1.15 (0.59-2.24)	0.67	
	GG	11 (13.7%)	10 (12.5%)	1.057 (0.593-2.063)	0.757	
	GG vs	11 (13.7%)	10 (12.5%)	1.11 (0.44-2.79)	0.81	
	AG + AA	69 (86.3%)	70 (87.5%)			
	AA vs	33 (41.3%)	36 (45%)	1.16 (0.62-2.17)	0.63	
	AG + GG	47 (58.7%)	44 (55%)			
	Allele					
rs2981582	T	89 (55.6%)	112 (70%)	2.925 (1.864-4.633)	**0.000**	**-**
	C	71 (44.4%)	48 (30%)	-	-	
rs1219648	A	102 (63.8%)	106 (66.3%)	-	-	
	G	58 (36.2%)	54 (33.7%)	0.860 (0.554-1.335)	0.501	

Table 4. Investigating the association between haplotypes of two SNPs studied in two groups and the risk of breast cancer

Haplotype	Chi-square	DF	P value
AC	8.205	1	0.004
GC	5.117	1	0.024
AT	11.809	1	0.000
GT	4.56	1	0.033
Total	21.914	3	0.000

DF: Degree of freedom

FGFR2 Gene Expression Analysis

Data on the expression of *FGFR2* from GEO datasets and Expression Atlas indicated a higher expression in lobular carcinoma samples than in healthy tissue, while they showed lower expression in ductal carcinoma samples. The difference was statistically significant (P=0.000 for lobular vs control; P<0.05 for ductal vs control).

DISCUSSION

Breast cancer is a multifactorial disorder shaped by both environmental and genetic factors and continues to be the most prevalent malignancy among women in Iran (5). Investigating the influence of genetic polymorphisms on cancer susceptibility is essential for enhancing early detection, risk assessment, and tailored treatment approaches. Polymorphisms in the *FGFR2* gene, specifically rs2981582 and rs1219648, are among the most extensively researched genetic markers in this area.

In the current research, we examined these two *FGFR2* polymorphisms in an Iranian female cohort and found a significant association between the TT genotype of rs2981582 and increased risk of breast cancer (P=0.001; OR=3.566). In contrast, rs1219648 was not significantly associated with the disease. Notably, haplotype analysis demonstrated a significant relationship between specific haplotypes and susceptibility to Breast cancer, indicating a potential synergistic effect of these SNPs on disease risk.

Much research has looked at the link between *FGFR2* SNPs, especially rs2981582 and rs1219648, and the risk of breast cancer in different groups of people. Most of these studies have found similar results; however, certain differences are important for each group. Jia et al. performed a meta-analysis of over 50,000 participants and established that rs2981582 and rs1219648 were significantly linked to the susceptibility of breast cancer in Caucasian and Asian populations (6). Zhang et al. also established that several intronic SNPs, especially rs2981582, were more strongly correlated when comparing East Asian individuals with Europeans due to the possibility of variations in the frequency of alleles as well as linkage disequilibrium structure (7). The results indicate a significant correlation between the rs2981582 TT genotype and heightened breast cancer susceptibility in Iranian women. This aligns with regional studies, including Zhang et al. (2016), which identified rs2981582 as a significant *FGFR2* variant associated with breast cancer in a pooled analysis of more than 35 studies (2). Cui et al. noted that even within Asia, populations such as Chinese and Korean women exhibited variable risk estimates, highlighting the necessity for population-specific studies, such as the one conducted in Iran (8). Interestingly, we observed a discrepancy in our findings from some studies conducted in the U.S. and some studies conducted in European populations. For instance, Zanna et al. observed a potential survival benefit in male breast cancer patients linked to the rs2981582 mutation, suggesting a possible gender or subtype-specific effect (9). Similarly, Wang et al. indicated a modifying effect of hormone receptor status on the *FGFR2*-breast cancer association. With regard to rs1219648, our study found no significant association in isolation, though haplotype analysis did show that combinations involving this SNP (e.g., TG) significantly increased disease risk (10). This aligns with the findings of Yang et al. in Chinese populations, where individual SNP effects were modest but haplotypic combinations conferred higher predictive power (11). Differences between studies can be attributed to multiple factors: Genomic architecture by ethnicity and allele frequency differences, Differences in environmental exposure and lifestyle choices, including smoking and contraceptive medication use, Study design heterogeneity includes heterogeneity in sample size, use of different genotyping methods, and adjustment for confounding variables. The difference in hormone receptor subtypes may also contribute to the interaction with *FGFR2* expression pathways (10). Overall, these studies have shown that rs2981582 is

robustly and consistently associated with breast cancer risk globally and exemplifies how local haplotype-based analysis is needed with underrepresented populations such as women of Iranian origin.

The molecular basis for the link between *FGFR2* polymorphisms and breast cancer has been partly explored. *FGFR2* is a member of a receptor family exhibiting tyrosine kinase activity, implicated in cell proliferation and differentiation, and encodes a receptor that executes this function. Single nucleotide polymorphisms (SNPs), including rs2981582, are found in intron 2 of the *FGFR2* gene - a region shown previously to harbor cis-regulatory elements - and these polymorphic variations are thought to enhance the binding affinity of transcription factors, including Oct-1, Runx2, and CP2, and upregulate *FGFR2* expression in breast epithelial cells (1). This dysregulation may promote mitogenic signaling, resistance to apoptosis, and eventually carcinogenesis.

Our gene expression analysis with publicly available datasets indicated that *FGFR2* was overexpressed in lobular carcinoma subtypes, while it was downregulated in ductal carcinoma. This finding highlights the heterogeneity of breast cancer and suggests that *FGFR2* expression may be restricted to certain subtypes. These patterns have already been described in other regional series, including the Markazi province study (12), which also highlighted the inconsistent risk conferred by *FGFR2* based on the subtype of tumor.

In clinical practice, identification of high-risk genotypes, especially rs2981582 TT and their associated risk haplotypes, can provide useful opportunities for the creation of population-specific genetic screening programs, especially in high-incidence populations of world regions such as Iran for early-onset breast cancer. *FGFR2* has also been investigated as a potential target for therapy, and the pattern of expression can aid in predicting the response to tyrosine kinase inhibitors or chemotherapy regimens (13).

Limitations: The sample size (n=160) was modest, potentially limiting statistical power for detecting smaller effects. The study did not assess hormone receptor subtypes (ER/PR/HER2), which may influence *FGFR2* associations. Lack of a replication cohort limits generalizability.

Future Directions: Larger studies, functional assays (e.g., luciferase assays for rs2981582), and integration of transcriptomic/epigenetic data are needed to elucidate *FGFR2*'s role in Iranian breast cancer patients.

CONCLUSION

This study suggests a strong association between the TT genotype of *FGFR2* rs2981582 and an increased risk of Breast cancer among women in Iran. Additionally, the rs1219648 genotype was effective at modulating the risk via haplotype. All of these results are consistent with those seen internationally and emphasize the role of the *FGFR2* variants in breast cancer predisposition. Because the intronic SNPs potentially possess regulatory function, this research validates the use of *FGFR2* genotyping as a population-level biomarker for early screening and risk, specific to the given population. Future studies ought to explore further the functional analyses and the role that the functional analyses play, besides clinical and hormonal parameters, to enhance precision oncology, especially in the local context.

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Ethics Approval and Consent to Participate

The study protocol was approved by the Ethics Committee of Arak University under the ethical approval code: IR.ARAKMU.REC.1395.288. Informed consent was obtained from all participants prior to inclusion in the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for Publication

Not applicable.

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