Journal of the Medical Research Institute

JMRI, 2025; Vol. 46 No.1: (36-46)

Journal of the Medical Research Institute

Association between Fibroblast Growth Factor Receptor 2 (FGFR2) Gene Polymorphism (rs2981582) and the Risk of Breast Cancer in Egyptian women.

Noha Said Kandil[1]*, Mohammad Fakry Elnemr[2], Heba Morsy Abd Elkader Morsy[3], eman mahrous[1], Mona Mohamed Abaza[1].

1) Department of Chemical Pathology, Medical Research Institute, Alexandria University, Alexandria, Egypt.
2) Department of Cancer Management and Research, Faculty of Medicine, Alexandria University, Alexandria, Egypt.
3) Department of Human Genetics, Faculty of Medicine, Alexandria University, Alexandria, Egypt

ABSTRACT:
Breast cancer is most frequently diagnosed malignancy among women representing a quarter of all cancers. FGFR2 is one of low penetrance genes. FGFR2 gene has a role in development and progression of BC.The SNP rs2981582 associated with a higher level of FGFR2 transcription both in cell lines and in tumors.
The aim of this work was to study the association between the rs2981582 and breast cancer risk in Egyptian women.
The studied subjects were classified into 50 apparently healthy volunteers and 50 of BC cases. Genomic DNA was extracted from peripheral blood leukocytes by using ion-exchange column chromatography. Genotyping was done using an allelic discrimination real-time PCR using TaqMan probes technology.
The present study revealed that, the genotype distribution pattern of rs2981582 was statistically significant between cases and controls. Both of heterozygous AG and mutant homozygous genotype AA showed higher frequency in cases while the wild homozygous GG showed higher frequency in controls. Heterozygous genotype was associated with increased breast cancer risk. The minor allele A showed higher frequency in cases, and it was associated with increased breast cancer risk.
Furthermore, the minor allele A showed a higher frequency in ER, PR positive patients, Luminal A tumors, positive lymph node involvement and patients with positive family history.
In conclusion: the present work demonstrated that the minor allele of rs2981582 of FGFR2 gene is associated with increased breast cancer risk in Egyptian women.

Keywords: FGFR2, breast cancer, SNP rs2981582

1. INTRODUCTION

Cancer breast represents is the most common cancer worldwide and the principal reason of fatality between females (Aglan et al., 2024); El-Attar et al. (2020). In Egypt, breast cancer represents 32 % of all cancers among Egyptian female patients (Ibrahim et al., 2014). It is a complex heterogeneous disease, caused by abnormal growth and uncontrolled cell proliferation within the terminal duct and lobules of the breast (Mahdi et al.,2013).Breast tumorigenesis comprises a multiplexed interaction of genomic, epigenetic, ecological along with lifestyle aspects. Genetic predisposition provides a crucial influence to the development of breast cancer doubling the risk in first degree relatives of affected women (Aglan et al., 2024; Kandil et al., 2022; Siddiqui et al., 2014).

Despite that, most of breast cancer patients are sporadic, an evident percentage of cases occurs as a result of highly penetrating genetic mutations in susceptibility genes. One of the best prognosticators of breast cancer is family history that anticipated the increased risk (Feng et al., 2018; Foulkes, 2008).

Mutations in BRCA1 and BRCA2 that impair gene function are the most significant genetic contributors to breast cancer risk, with carriers having up to an 85% lifetime likelihood of developing the disease (Feng et al., 2018; Metcalfe et al., 2010).

High-risk genetic mutations play an important role but represent not more than 25% of familial breast cancer risk and less than 5% of overall susceptibility, as they are uncommon in the general population. (Shen et al., 2021; Thompson & Easton, 2004). It is proposed that the enduring risk could be caused from a mixture of several frequent mutations, independently presenting a modest influence on the risk of development of cancer breast (Antoniou & Easton, 2006).

Association between Fibroblast Growth Factor Receptor 2 (FGFR2) Gene Polymorphism (rs2981582) and the Risk of Breast Cancer in Egyptian women.
Received: 15-12-2024 Accepted: 20-3-2025
Corresponding author: **Noha Said Kandil**

A number of common low-penetrance genomic mutations related to predisposition of the development of cancer breast have been identified, however their impact to tumorigenesis varies among populations (Pal et al., 2024).

Single-nucleotide polymorphisms are the commonest type of genetic mutations, it can cause silent, harmless or harmful effects, SNPs take place in either coding or non – coding areas, but mostly in the non-coding regions. The genetic profiles of SNPs can help recognize the full range of genes related to the development of compound disorders such as cancer breast (Deng et al., 2017; Ledwoń et al., 2013).

Breast cancer is associated with genetic variants in critical genes and protein pathways. It has been identified that fibroblast growth factor receptor 2 (FGFR2), a gene involved in mammary gland development, as a promising gene for breast cancer risk in various populations (Sumbal & Koledova, 2019; Sun et al., 2010).

Fibroblast growth factor receptor 2 is part of the FGFR group of tyrosine kinase receptors. Its gene is located at chromosome 10q26, encoding FGFR2b and FGFR2c isoforms due to alternative splicing (Sumbal & Koledova, 2019). They function as fibroblast growth factor (FGF) receptors transducing FGF signals through fibroblast growth factor receptor substrate (FRS2), and through phospholipase C gamma (PLCγ) (Sumbal & Koledova, 2019). They may contribute to the course of carcinogenesis via promotion of the growth of cancer cells, increasing its spreading properties, enhancing migration and formation of new vascular supply to the tumor (Ardizzone et al., 2023).

Many SNPs in FGFR2 were studied as a risk of breast cancer. The most significantly associated SNP is rs2981582 which lies within the intron 2 of FGFR2. Intron 2 of FGFR2 contains several putative transcription-factor binding sites, some of them lie near the relevant SNPs. So, the association with breast cancer risk is mediated through regulation of FGFR2 expression (Easton et al., 2007).

Genetic variations, such as the differences in SNP allele frequencies across different ethnic groups, can influence breast cancer occurrence and features. SNPs linked to risk in one population may not have the same effect in others, emphasizing the need for global replication studies across diverse populations (Siddiqui et al., 2014).

The mutant (A or sometimes referred to as T) allele of FGFR2 rs2981582 was associated with upregulation of FGFR2 and increased expression in cancer breast and acts as a considerable risk factor for oncogenesis. But this was subjected to high degree of variability due ethnic diversity resulting in contradictory results in different studied populations (Shu et al., 2019).

The association of FGFR2 polymorphisms with breast cancer has also been reported in Asians (Lin et al., 2012; Siddiqui et al., 2014), African-American, and European-American women (Rebbeck et al., 2009). This research aims at studying the association between FGFR2 polymorphism (rs2981582) and breast cancer risk in Egyptian population.

Subjects and Methods

The study was approved from the ethical committees of the Medical Research Institute, Alexandria University; with approval number .the study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. Informed consent was obtained from all eligible subjects before starting the study. The study included 100 subjects divided into 50 apparently healthy female volunteers and 50 female patients histopathologically diagnosed as primary BC. The patients were recruited from the surgery department of Medical research institute, Alexandria University. The inclusion criteria for the subjects in the present study include treatment naïve female patients diagnosed with primary breast cancer without any exposure to either chemotherapy or radiotherapy and not metastasizing.

Methods

Specimen preparation and storage:

After an eight hours fasting period, five milliliters venous blood were withdrawn from each subject and divided into 3 milliliters of whole blood dispensed into a plain serum vacutainer tube and was allowed to clot for 30 minutes then centrifuged for ten minutes at 4000 rpm. The obtained serum was immediately separated and used for routine biochemical tests. Another 2 milliliters of whole blood were dispensed into a vacutainer tube coated with potassium salt of ethylene diamine tetra acetic acid (K3 EDTA) and mixed by gentle inversion for genomic analysis.

Laboratory investigations

A. blood glucose (FBG),Urea, creatinine, alanine Fasting aminotransferase (ALT) and aspartate aminotransferase (AST): (Burtis et al., 2011)

These assays were performed on Olympus AU400 Chemistry Auto analyzer, where the final absorbance of (fasting serum glucose, urea, and creatinine) and delta absorbance of (GGT, ALT, and AST) were compared to standard (auto calibrator) of known concentration.

B. FGFR 2 SNP rs2981582 genotyping study: (Chen, 2016)

a. DNA extraction:

Genomic DNA extraction from leukocytes was done using PureLink® Genomic DNA Mini Kit (Invitrogen) according to the manufacturer's instructions using the ion exchange chromatography property of the provided columns.

b. DNA Quantification and purity:

The concentration and purity of the extracted genomic DNA was determined using the Thermo Scientific NanoDrop™ 1000 Spectrophotometer. Purity was assessed by absorbance at 230, 260 and 280 nm. The ratio of absorbance at (260/280) ~1.8 were accepted as "pure" for DNA. The ratio 260/230 in the range of 2.0-2.2 were accepted as "pure" for DNA.

C. Real Time Polymerase Chain Reaction FGFR 2 single nucleotide gene polymorphism rs2981582 genotyping

Genotyping of FGFR 2 rs2891582 was performed by the allelic discrimination Real-Time Polymerase Chain Reaction SNP genotyping technology using dual labelled fluorogenic TaqMan® minor groove binder (MGB) probes. It begins with DNA denaturation at high temperature then two pre-designed primers (forward and reverse) complementary to the 3' ends of the sequence are annealed at lower temperature and then extending at an intermediate temperature by the heat stable Thermus aquaticus (Taq) DNA polymerase enzymatic activity. The PCR process is based on the repetition of this cycle and can amplify DNA segments by at least 10^5fold. The

thermal cycling conditions of the (Eco- ilumina real time PCR, USA) were programmed as shown in (Table 1).

The TaqMan SNP Genotyping assay is a 5'-nuclease assay that uses 5'-nuclease activity of the Taq DNA polymerase for SNP genotyping, the following steps occur:

1. The forward and reverse PCR primers anneal specifically to their complementary sites that include the SNP polymorphic site in between.
2. Probes, anneals specifically to its complementary sequence between the forward and reverse primer sites
3. Extension of the primers bound to the template DNA, then polymerase displaces then cleaves the probes resulting in an increase in fluorescence by this reporter.
4. The mismatching probe will consequently have a lower melting temperature and consequently no fluorescence signal would be detected from this probe. (Table 2)

Table (1): The thermal cycling conditions

Step	AmpliTaq Gold Enzyme Activation	PCR (40 Cycles)	
		Denature	Anneal/Extend
Temperature	95 °C	92 °C	60 °C
Time	Hold for 10 minutes	15 sec	1 min

Table (2):The rs2981582 SNP genotyping according to the fluorescence signals of the sample

A substantial increase in	Indicates	Genotyping
VIC-dye fluorescence only (VIC / VIC	Homozygosity for A allele (Homo-mutant genotype)	AA
FAM-dye fluorescence only (FAM/FAM)	Homozygosity for G allele (Homo-wild genotype)	GG
Both VIC- and FAM-dye fluorescence (FAM/ VIC)	Allele A -Allele G (heterozygosity)	AG

Statistical analysis

Data was studied by means of IBM SPSS version 20.0. The normality of distribution was evaluated using Kolmogorov-Smirnov test. The categorical variables were compared using Chi-square test (Monte Carlo). The Mann-Whitney test was employed to compare two groups with non-normally distributed quantitative variables, while ANOVA was used for group comparisons, followed by a Post Hoc test (LSD) for pairwise analysis. The Kruskal-Wallis test was applied for multi-group comparisons of non-normally distributed quantitative variables, followed by Dunn's multiple comparisons test for pairwise analysis. The odds ratio (OR) and 95% confidence interval were calculated to determine the likelihood of an event occurring in the risk group compared to the non-risk group. Statistical significance was set at a 5% level. The allele frequency distribution at each polymorphism locus was tested against the Hardy-Weinberg Equilibrium.

Results

Most of patients (98%) were diagnosed as IDC while only (2%) of them were ILC. Almost one third of them were classified as T2 (33%). Regarding Lymph node involvement and distant metastasis, most of patients were positive for lymph node (68%) while most of them were negative for distant metastasis (86%). Stage II and stage III were of the highest frequency each one of them was (42%). For biological tissue markers, most of cases were positive for ER (80%) and PR (74%) however (66%) of cases were negative for HER2. Regarding clinical data, Most of patients (70%) had a negative family history, while the frequency of menopausal patients was higher (58%). The observed genotype frequency was in consistency with Hardy Weinberg equilibrium of population in both cases (p=0.145) and controls (p=0.335)**. (Table 3)**

Table (3): Clinicopathological data of the studied BC patients

	No.	**%**
Histopathology		
IDC	49	98.0
ILC	1	2.0
Tumor		
T1	3	6.0
T2	33	66.0
T3	10	20.0
T4	4	8.0
Lymph node		
Negative	16	32.0
Positive	34	68.0
Metastasis		
No	43	86.0
Yes	7	14.0
Stage		
I	1	2.0
II	21	42.0
III	21	42.0
IV	7	14.0
ER		
Negative	10	20.0
Positive	40	80.0
PR		
Negative	13	26.0
Positive	37	74.0
HER2		
Negative	33	66.0
Positive	17	34.0
Family history		
Negative	35	70.0
Positive	15	30.0
Menstrual History		
Menstruating	21	42.0
Menopausal	29	58.0

The observed minor allele frequency (A) of rs2981582 was (24%) in cases while in controls it was (12%). There was a statistically significant increase of minor allele frequency (A) of rs2981582 in cases than controls, (p=0.027). The Minor allele (A) was found to be associated with a statistically increased risk of breast cancer with an Odds ratio (2.316) (95%C.I. = [1.08 - 4.94]). There was a statistically significant difference of rs2981582 genotype distribution between cases and controls, (p=0.036) with an increased breast cancer risk in case of heterozygous genotype AG with an Odds ratio (2.358) (95%C.I. = [1.09 – 6.09])**. (Table 4)**

Table (4):Comparison between the studied groups according to genotype distribution and Allele frequency of the rs2981582.

	Control (n= 50)		Cases (n= 50)		p	OR	C.I
	No.	%	No.	%			
Genotyping							
GG	38	76.0	27	54.0	$^{MC}p=$ 0.036*	1.000	
AG	12	24.0	22	44.0		2.580	1.09 – 6.09
AA	0	0.0	1	2.0		-	
Allele frequency							
G	88	88.0	76	76.0	0.027*	1.000	
A	12	12.0	24	24.0		2.316	1.08 – 4.94

p: p values for Chi square test for comparing between the two groups
^{MC}p: p value for Monte Carlo for Chi square test for comparing between the two groups
*: Statistically significant at $p \leq 0.05$

The frequency of the variant (AA) genotype was 2% in the cases and zero percent in the control group. The mutant allele resulting from the SNP alters either function or expression of the FGFR2 protein, causing abnormal downstream signal transduction that ultimately causing cancer breast (Yasam, 2023)

Assuming a dominant mode of inheritance, where the exposed group was composed of subjects with both the AA and AG genotypes, the genotype distribution showed a statistically significant difference (p=0.021) between cases and controls. Also, regarding this mode of inheritance, genotype distribution was found to be associated with a statistically increased risk of breast cancer with an Odds ratio (2.697) (95%C.I. = [1.15 – 6.34]).

On the other hand, assuming a recessive mode of inheritance, where the exposed group was composed of only subjects with the homo-mutant AA genotype, the genotype distribution showed no statistically significant difference between cases and control.

On assuming an additive mode of inheritance, there was a statistically significant difference between cases and controls (p=0.036) in genotyping distribution.

The minor allele (A) frequency was increased among cases with ER positive (83.3 %) and PR positive tumors (70.8%), while it was higher in HER2 negative tumors (62.5%).

There was a statistically significant difference of rs2981582 genotype distribution between cases and controls, (p=0.036) with an increased breast cancer risk in case of heterozygous genotype AG with an Odds ratio (2.358) (95%C.I. = [1.09 – 6.09]). On assuming both the dominant and additive mode of inheritance, the genotype distribution showed a statistically significant difference between cases and controls, (p=0.021), (p=0.036) respectively, with a statistically increased risk of breast cancer with an Odds ratio (2.697) (95%C.I. = [1.15 – 6.34]).On the other hand, assuming a recessive mode of inheritance, the genotype distribution showed no statistically significant difference between cases and control. (Table 4).

The minor allele (A) frequency was increased among cases with ER positive (83.3 %) and PR positive tumors (70.8%), while it was higher in HER2 negative tumors (62.5%), (Table 5, 6).

Table (5): Comparison between the two studied groups according to mode of inheritance

Mode	Control (n= 50)		Cases (n= 50)		p	OR	C.I
	No.	%	No.	%			
Recessive							
GG +AG	50	100.0	49	98.0	$^{FE}p=$ 1.000	1.000	
AA	0	0.0	1	2.0		-	-
Dominant							
GG	38	76.0	27	54.0	0.021*	1.000	
AG +AA	12	24.0	23	46.0		2.697	1.15 – 6.34
Additive							
GG	38	76.0	27	54.0	$^{MC}p=$ 0.036*	1.000	1.09 - 6.09
AG	12	24.0	22	44.0		2.580	
AA	0	0.0	1	2.0			

p: p values for Chi square test for comparing between the two groups
^{FE}p: p value for Fisher Exact for Chi square test for comparing between the two groups
^{MC}p: p value for Monte Carlo for Chi square test for comparing between the two groups
*: Statistically significant at $p \leq 0.05$

Table (6): Association between Allele frequency and biological tissue markers in cases

	Allele frequency				**p**
	G (n= 76)		**A (n= 24)**		
	No.	**%**	**No.**	**%**	
ER					
Negative	16	21.1	4	16.7	^{FE}p= 0.775
Positive	60	78.9	20	83.3	
PR					
Negative	19	25.0	7	29.2	0.685
Positive	57	75.0	17	70.8	
HER2					
Negative	51	67.1	15	62.5	0.678
Positive	25	32.9	9	37.5	

p: p values for **Chi square test** for comparing between the two groups
^{FE}p**:** p value for **Fisher Exact** for Chi square test for comparing between the two groups

Discussion

Several risk factors contribute to BC risk (Kamińska, 2015). Advances in the screening methods, early diagnosis, and treatment led to improved prognosis and increased survival. Moreover, the breakthrough in biomolecular science aided in deeper insight of development of cancer breast, allowing the development of advanced treatment remedies capable of targeting cancer and react effectively to its microenvironment (Nounou, 2015).

Hereditary breast cancer is mainly related to mutations in BRCA1 and BRCA2 genes which represent the majority of high penetrance genetic BC (Mehrgou, 2016, Neamatzadeh, 2015, Mahdavi, 2019). Regarding low penetrance genes, genome-wide association studies have identified a low-risk panel of different alleles and loci including FGFR2. The relative risk of BC by any one of the genetic variants alone is trivial, however, interplay between such genes and other higher and low risk genes, improves their clinical relevance (Turnbull, 2008). Fibroblast Growth Factor Receptor 2 has a significant effect in the development of mammary gland. It has been implicated in development and progression of BC (Ardizzone, 2023). In BC patients, increased both nuclear and cytoplasmic FGFR2 expression correlated with lower overall and disease-free survival (Sun, 2012). Dysregulated FGF signaling has been linked to the development and progression of various cancers. (Wiedlocha, 2021, Ahmad, 2012, Moffa, 2004). Genome wide association study has identified multiple SNPs in intron 2 of the FGFR2 gene associated with a higher risk of BC. The SNP rs2981582 has been identified as the most strongly associated SNP with breast cancer (Easton, 2007, Shu, 2019). It has been demonstrated that the minor allele of rs2981582 is linked to higher FGFR2 transcription levels in both cell lines and tumors (Meyer, 2008).

In the current study the frequency of the variant (AA) genotype was 2% in the cases and zero percent in the control group. This was opposed by findings reported by Jahan et al., (2023) who stated that the homozygous mutant allele among the cases group was 22% as compared to 12% in the control group. This variation in the frequencies could be related to small sample size and different ethnic group of the studied population. However, they too reported increased frequency of the mutant allele among the cases group as we did. There was a statistically significant difference between controls and cases regarding genotype distribution (^{MC}p=0.036) with an increased breast cancer risk with an Odds ratio (2.58) as seen with AG genotype, (Table 5).

In accordance with our study, previous studies have reported the association between SNP rs2981582 and breast cancer in different population. (Huijts, 2007), (McInerney, 2009), (Shan, 2012), (Jara, 2013) and (Siddiqui, 2014) reported that, the rs2981582 minor allele is associated with increased BC risk with an Odds ratio (1.3), (1.22), (2.0), (1.37) and (1.29) respectively. In Tunisian women it was reported that, the observed minor allele frequency was (50.2%) which showed a statistically significant difference (p=.000004) between cases and controls. It was associated with increased BC risk with an Odds ratio (2.0). In north India, it was reported that, the rs2981582 observed minor allele frequency was (38.04%) in cases compared to (32.1%) in controls which showed a statistically significant difference (p=0.011). The minor allele was associated with increased BC risk with an Odds ratio (1.29). Genotype distribution showed a statistically significant difference (p=0.045) between cases and controls. In cases genotype frequency was observed as follows (GG=39.1%, AG= 45.6% and AA= 15.2) whereas in controls it was as follows (GG= 46.6%, AG= 42.4% and AA= 10.9). An increased BC risk with an Odds ratio (1.66) was observed with homozygous genotype AA. In Pakistani women it was shown that, the rs2981582 genotype distribution pattern was as follows; the wild homozygous genotype GG was higher in controls (20%) than cases (5%), for heterozygous genotype AG was higher in cases (91%) compared to controls (76%)

and the mutant homozygous showed equal frequency in cases and controls (4%). The heterozygous genotype AG was significantly associated with breast cancer (p=0.001) with an Odds ratio (1.19) (95% CI 1.07 – 1.34) (38). The association between FGFR 2 SNPs and breast cancer risk remains inconclusive due to differences in ethnic and regional and other factors. The frequency of the mutant homozygous allele among Egyptian patients suffering from HCC was 16% compared to 10% among the control group, as reported by Al-Khaykanee et al., (2021) However, due to insufficient literature regarding the allele frequency among Egyptian patients suffering from breast cancer, we could not compare our findings with other similar studied population.

When FGFs bind to their receptors, the functional domains within the receptors stimulate tyrosine kinase activity, leading to phosphorylation of the receptor. This initiates a sequence of signaling pathways, including RAS-MAPK, STATs, and PLC-γ, which control the subsequent gene expression. These genes influence various biological and disease-related conditions, such as cell apoptosis, metastatic behavior, proliferation, differentiation, and new vessel formation (Wesche, 2011).

SNPs in FGFR 2 gene, rs2981582 represents one of the robust and most coherent genetic predisposition for breast cancer (Hunter, 2007). The hazard occurs to be coherent in Caucasian (Huijts, 2007, Boyarskikh, 2009, Rebbeck, 2009, Hemminki, 2010), Asian (Liang, 2008), Hispanic women living in the US (Slattery, 2011). Nevertheless, to our knowledge, no previous studies regarding the FGFR2 SNP rs2981582 and the risk of breast cancer in Egyptian women were done.

Regarding the FGFR2 gene polymorphisms, there is in vitro evidence that the mechanism might be more related to an anti-apoptotic than to a mitogenic effect (Hishikawa, 2004, Tamaru, 2004). The suppression of programmed cell death may be facilitated via the downregulation of Forkhead O transcription factors (FOXO) synthesis. FOXO proteins play key roles in inducing apoptosis, repairing DNA damage, detoxifying reactive oxygen species (ROS), and suppressing the transcriptional activity of estrogen receptors ERα and ERβ. FGFR2 activates the PI3K signaling pathway, an intracellular system that inhibits the transcription of FOXO factors (Kandil, 2022, Yang, 2009).

Meyer et al, (Meyer, 2008) reported that minor allele of rs2981582 is linked to increased transcription of FGFR2 not only in cell lines but also in tumor cells. Increased FGFR2 gene expression contributes to the process of tumorigenesis through cell growth, invasiveness, motility and angiogenesis. It remains necessary to discover the concrete mechanisms about the phenotypic outcome of impaired FGF signaling caused by FGFR2 gene SNPs in breast cancer.

On the other hand, few studies failed to confirm the association between FGFR2 SNP rs2981582 and breast cancer occurrence, one of them was reported in Chinese women of the Han nationality, it was reported that rs2981582 is not a risk factor for BC with an Odds ratio for AG (0.99) (95% CI 0.73–1.33) and AA (0.85) (95% CI 0.54–1.33). Genotype distribution pattern was (GG=34%, AG=54% and AA=12% in cases) and (GG=33%, AG=53% and AA=14% in controls), which showed no statistically significant difference (p=0.99) between cases and controls. The minor allele frequency in cases was (61%) compared to (%59) in controls, which showed no statistically significant difference (p=0.568) between cases and controls (Chen, 2012).

Another study was done by Chen (2016) reported that, the minor allele of rs2981582 had a reduced breast cancer risk (GA vs GG: OR = 0.444, 95 % CI = 0.262–0.752; AA vs GG: OR = 0.579, 95 % CI = 0.342–0.983) (Chen, 2016).

The most likely explanation for such differences is the sample size examined as well as broad changes in the genetic landscape, including variations in allele frequencies of SNPs and disparities among ethnic groups.

Another reason may be using different technique to determine the SNP which differs in method performance.

In the present study, the BC risk was assessed according to different modes of inheritance of FGFR2 SNP rs2981582 genotypes. When applying the dominant and additive modes of inheritance the variant genotype was significantly higher among cases, (p=0.021), (p=0.036) respectively, with increased risk of breast cancer with an Odds ratio (2.697) (95%C.I. = [1.15 – 6.34]). Contrarily, the recessive mode of inheritance didn't show differences between cases and control. The mutant allele arising from the SNP modifies the function or expression of the FGFR2 protein, resulting in abnormal downstream signal transduction and ultimately leading to breast cancer (Yasam, 2023).

Our results were consistent with previous studies, (Liang, 2008), (Han, 2011), (Jara, 2013), and (Siddiqui, 2014) reported that, on assuming a dominant mode of inheritance, the genotype distribution (AG+AA) showed a statistically significant difference (p=0.0003), (p=0.0047), (p=.0007) and (p=0.025) between cases and controls and a an increased the risk of breast cancer with an Odds ratio (1.36), (1.15), (1.61) and (1.37) respectively.

Although the minor allele did not show a significant difference with ER or PR status, but its frequency was higher among ER positive patients (83.3%) as well as patients with PR positive (70.8%). This could be attributed to the small sample. Many studies reported significant association between the minor allele frequency and ER, PR status in many populations. (Garcia-Closas, 2008) reported that FGFR2 rs2981582 was more strongly related to ER-positive (per-allele OR (95%CI)=1.31 (1.27–1.36)) in European and Asian participants. (Slattery, 2011) reported that rs2981582 was associated with almost a twofold increased risk of an ER+/PR+ tumor, while not-significantly associated with ER−/PR− tumors. (Liang, 2008) stated that the genotype variant A was strongly associated with ER/PR-positive tumors. Another study by (Siddiqui, 2014) reported that the minor allele displayed a stronger association with ER-positive women (TT vs. CC, P = 0.001; CT+TT vs. CC, P = 0.012).

This may be explained by many reasons; elevated FGFR2 expression levels have been observed in ER-positive cell lines and tumors compared to ER-negative ones (Tozlu, 2006). This may be due to presence of ER binding sites within the FGFR2 gene. Increased concentrations of sex

hormones, particularly estrogens, can augment the risk of development of cancer breast. Factors related to menstrual cycle and reproduction, such as early menarche and delayed initial childbirth, that could be signs of endogenous sex hormones levels, are considered as significant predisposing factors for the development of cancer breast. Progesterone receptor can be directly stimulated via the Mitogen-activated protein kinase (MAPK) pathway independently from their respective ligands, which can be triggered by FGFR2 (Qiu, 2003). Furthermore, the FGF2/FGFR2 axis may contribute to PR stimulation, driving hormone-independent growth of mammary tumor, probable through the stimulation of the MAPK pathway (Giulianelli, 2008).

The minor allele showed the highest frequency (45.8%) in Luminal A type compared to other molecular types, but it did not reach a statistically significant difference which may be caused by the small sample size. Broeks et al, (2011) stated that the rs2981582 had a strongest association with luminal tumors. This finding could be explained by the association of FGFR2 SNP rs2981582 with ER and PR positive tumors (Broeks, 2011).

In the present study there was a statistically significant difference between cases and controls regarding the BMI (p=0.001). Previous study reported that ORs were slightly higher for women with a BMI > 30 kg/m2 compared to women with BMI < 30 kg/m2 (Marian, 2011).

This finding could be explained by the increased BMI is a risk factor for BC (Carpenter, 2003). Fat tissue serves as the primary source of estrogen synthesis, in obese postmenopausal females, through aromatase, that is present in both adipose tissue in the breast and in the tumor tissue itself. Androgens formed from the adrenal cortex and ovaries of postmenopausal females, are metabolized into estrogens by aromatase. This local estrogen production can result in estrogen levels in cancer breast which can be higher up to 10 times than those in the bloodstream (Cleary, 2009).

In the present study, the minor allele (A) frequency was higher in menopausal cases (66.7%) but it did not reach a statistically significant difference, (Table 3). Rebbeck et al, (Rebbeck, 2009) reported that a significant association was found between the rs2981582 and breast cancer in post-menopausal women with an Odds ratio (1.26 (95% CI: 1.04–1.53).

Increased minor allele frequency in postmenopausal breast cancer is considered to be related to increase in the circulating estrogen levels. In postmenopausal women, the major source of estrogen is adipose tissue (Rebbeck, 2009).

On the other hand, most epidemiological studies showed an association with premenopausal women. Siddiqui et al, (Siddiqui, 2014) reported an association of the rs2981582 with premenopausal women with breast cancer. Another study stated a strong association between rs2981582 and breast cancer in premenopausal women (Liang, 2008). Ozgoz et al, (Ozgöz, 2013) observed that, the rs2981582 mutant homozygous genotype was more frequent in premenopausal (45.5%) breast cancer patients.

In the current study, the minor allele frequency was higher in cases with positive lymph nodes involvement (75%) which did not show a statically significant difference. This finding may be attributed to the small sample size. previous study reported that the rs2981582 mutant homozygous genotype frequency was higher in patients with lymph node-positive tumors (31.8%) (Ozgöz, 2013). Another study reported that the association of rs2981582 with breast cancer risk tended to be stronger for patients with positive (per-allele OR (95% CI)=1.33 (1.27–1.39) compared to negative (1.25 (1.20–1.29)) nodal involvement (Garcia-Closas, 2008).

In conclusion the present work demonstrated that the minor allele of rs2981582 of FGFR2 gene is associated with increased breast cancer risk in Egyptian women. Also, it showed a higher frequency in ER positive patients, PR positive patients, Luminal A tumors, positive lymph node involvement and patients with positive family history. Finally, FGFR2 genotyping, particularly rs2981582, offers potential for enhancing breast cancer screening and risk stratification in Egypt. Identifying carriers of the high-risk allele could support personalized screening, enabling earlier detection and targeted interventions for high-risk subgroups, particularly for women with a family history of breast cancer or other predisposing factors, to optimize early detection and preventive strategies.. This approach can refine national cancer screening protocols by integrating genetic risk, optimizing resource allocation. Furthermore, Egypt-specific data on FGFR2 polymorphisms could inform global breast cancer risk models. Successful implementation would require addressing cost, infrastructure, and ethical considerations, including genetic counseling and data privacy. Another important point is targeting FGFR2, the receptors, and downstream signaling molecules could be explored as possible targeted therapy for such patients.

Future research should focus on validating these findings across diverse populations to account for ethnic and regional variations in allele frequencies and their associations with breast cancer. Additionally, exploring interactions between multiple SNPs, such as those in FGFR2, BRCA1/2, and other low-penetrance loci, could uncover complex genetic contributions to breast cancer risk. Investigating the molecular mechanisms by which FGFR2 polymorphisms influence tumor biology, including interactions with hormonal receptors, could further elucidate pathways for targeted therapies. Large-scale, longitudinal studies examining the integration of FGFR2 genotyping into existing screening programs would also be valuable for assessing its clinical and economic feasibility.

List of abbreviations

HRT	:	Hormone replacement therapy
IDC	:	Infiltrating ductal carcinoma
ILC	:	Infiltrating lobular carcinoma
LCIS	:	Lobular in situ carcinoma
NGS	:	Next Generation Sequencing
PALB2	:	Partner and localizer of BRCA2 gene
PTEN	:	Phosphatase and tensin homolog gene
RFLP	:	Restriction fragment length polymorphism
S.D	:	Standard deviation
SSCP	:	Single Strand Conformation Polymorphism
TICs	:	Tumor-initiating cells
Tm	:	Melting temperature
TP53	:	Tumor protein 53

References

Aglan, S. A., Awad, A. M., Elwany, Y. N., Shawky, S., Salam, R. M. A., Omar, R. S., Ghazala, R. A. M., Soliman, N. A., Khedr, M. I., Kandil, L. S., Sultan, M., Hamed, Y., & Kandil, N. S. (2024). BECN1 mRNA expression in breast cancer tissue; significant correlation to tumor grade. *Molecular Genetics and Genomics*, *299*(1), 56. https://doi.org/10.1007/s00438-024-02145-2

Ahmad, I., Iwata, T., & Leung, H. Y. (2012). Mechanisms of FGFR-mediated carcinogenesis. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1823(4), 850-860. https://doi.org/https://doi.org/10.1016 /j.bbamcr.2012.01.004

Al-Khaykanee, A. M., Abdel-Rahman, A. A. H., Essa, A., Gadallah, A.-N. A.-A., Ali, B. H., Al-Aqar, A. A., Badr, E. A. E., & Shehab-Eldeen, S. (2021). Genetic polymorphism of fibroblast growth factor receptor 2 and trinucleotide repeat-containing 9 influence the susceptibility to HCV-induced hepatocellular carcinoma. Clinics and Research in Hepatology and Gastroenterology, 45(6), 101636. https://doi.org/ https://doi.org/10.1016/j.clinre.2021.101636.

Antoniou, A. C., & Easton, D. F. (2006). Models of genetic susceptibility to breast cancer. *Oncogene*, *25*(43), 5898-5905. https://doi.org/10.1038/sj.onc.1209879

Ardizzone, A., Bova, V., Casili, G., Repici, A., Lanza, M., Giuffrida, R., Colarossi, C., Mare, M., Cuzzocrea, S., Esposito, E., & Paterniti, I. (2023). Role of Basic Fibroblast Growth Factor in Cancer: Biological Activity, Targeted Therapies, and Prognostic Value. *Cells*, *12*(7), 1002. https://www.mdpi.com/2073-4409/12/7/1002

Burtis, C. A., Ashwood, E. R., & Bruns, D. E. (2011). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics - E-Book: Tietz Textbook of Clinical Chemistry and Molecular Diagnostics - E-Book*. Elsevier Health Sciences. https://books.google.com.eg/ books?id=BBLRUI4aHhkC

Broeks, A., Schmidt, M. K., Sherman, M. E., Couch, F. J., Hopper, J. L., Dite, G. S., Apicella, C., .., & Garcia-Closas, M. (2011). Low penetrance breast cancer susceptibility loci are associated with specific breast tumor subtypes: findings from the Breast Cancer Association Consortium. Hum Mol Genet, 20(16), 3289-3303. https://doi.org/10.1093/hmg/ddr228

Boyarskikh, U. A., Zarubina, N. A., Biltueva, J. A., Sinkina, T. V., Voronina, E. N., Lazarev, A. F., Petrova, V. D., Aulchenko, Y. S., & Filipenko, M. L. (2009). Association of FGFR2 gene polymorphisms with the risk of breast cancer in population of West Siberia. *Eur J Hum Genet*, *17*(12), 1688-1691. https://doi.org/10.1038/ejhg.2009.98

Carpenter, C. L., Ross, R. K., Paganini-Hill, A., & Bernstein, L. (2003). Effect of family history, obesity and exercise on breast cancer risk among postmenopausal women. Int J Cancer, 106(1), 96-102. https://doi.org/10.1002/ijc.11186

Chen, F., Lu, M., Xue, Y., Zhou, J., Hu, F., Chen, X., Zhao, Z., Li, Y., & Wang, X. (2012). Genetic variants of fibroblast growth factor receptor 2 (FGFR2) are associated with breast cancer risk in Chinese women of the Han nationality. Immunogenetics, 64(1), 71-76. https://doi.org/10.1007/s00251-011-0564-2

Chen, Y., Shi, C., & Guo, Q. (2016). TNRC9 rs12443621 and FGFR2 rs2981582 polymorphisms and breast cancer risk. World Journal of Surgical Oncology, 14(1), 50. https://doi.org/10.1186/s12957-016-0795-7

Cleary, M. P., & Grossmann, M. E. (2009). Minireview: Obesity and breast cancer: the estrogen connection. Endocrinology, 150(6), 2537-2542. https://doi.org/10.1210/en.2009-0070

Deng, N., Zhou, H., Fan, H., & Yuan, Y. (2017). Single nucleotide polymorphisms and cancer susceptibility. *Oncotarget*, *8*(66). https://www.oncotarget.com/article /22372/text/

Easton, D. F., Pooley, K. A., Dunning, A. M., Pharoah, P. D., Thompson, D., Ballinger, D. G., Struewing, J. P.,., & Ponder, B. A. (2007). Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature*, *447*(7148), 1087-1093. https://doi.org/10.1038/ nature05887

El-Attar, E., Kamel, A., Karmouty, A., Wehida, N., Nassra, R., El Nemr, M., & Kandil, N. S. (2020). Assessment

of Serum CoQ10 Levels and other Antioxidant Markers in Breast Cancer. *Asian Pac J Cancer Prev*, *21*(2), 465-471. https://doi.org/10.31557/apjcp.2020.21.2.465

Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., Ji, X., Liu, W., Huang, B., Luo, W., Liu, B., Lei, Y., Du, S., Vuppalapati, A., Luu, H. H., Haydon, R. C., He, T. C., & Ren, G. (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes Dis*, *5*(2), 77-106. https://doi.org/10.1016/j.gendis.2018.05.001

Foulkes, W. D. (2008). Inherited susceptibility to common cancers. *N Engl J Med*, *359*(20), 2143-2153. https://doi.org/10.1056/NEJMra0802968

Garcia-Closas, M., Hall, P., Nevanlinna, H., Pooley, K., Morrison, J., Richesson, D. A., Bojesen, S. E., Nordestgaard, B. G., .., & Pharoah, P. D. (2008). Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. PLoS Genet, 4(4), e1000054. https://doi.org/10.1371/journal.pgen.1000054

Giulianelli, S., Cerliani, J. P., Lamb, C. A., Fabris, V. T., Bottino, M. C., Gorostiaga, M. A., Novaro, V., Góngora, A., Baldi, A., Molinolo, A., & Lanari, C. (2008). Carcinoma-associated fibroblasts activate progesterone receptors and induce hormone independent mammary tumor growth: A role for the FGF-2/FGFR-2 axis. Int J Cancer, 123(11), 2518-2531. https://doi.org/10.1002 /ijc.23802

Hemminki, K., Müller-Myhsok, B., Lichtner, P., Engel, C., Chen, B., Burwinkel, B., Försti, A., Sutter, C., Wappenschmidt, B., Hellebrand, H., Illig, T., Arnold, N., Niederacher, D., Dworniczak, B., Deissler, H., Kast, K., Gadzicki, D., Meitinger, T., Wichmann, H. E., Kiechle, M., Bartram, C. R., Schmutzler, R. K., & Meindl, A. (2010). Low-risk variants FGFR2, TNRC9 and LSP1 in German familial breast cancer patients. Int J Cancer, 126(12), 2858-2862. https://doi.org/10.1002/ijc.24986

Hishikawa, Y., Tamaru, N., Ejima, K., Hayashi, T., & Koji, T. (2004). Expression of keratinocyte growth factor and its receptor in human breast cancer: its inhibitory role in the induction of apoptosis possibly through the overexpression of Bcl-2. Arch Histol Cytol, 67(5), 455-464. https://doi.org/10.1679/aohc.67.455

Huijts, P. E., Vreeswijk, M. P., Kroeze-Jansema, K. H., Jacobi, C. E., Seynaeve, C., Krol-Warmerdam, E. M., Wijers-Koster, P. M., Blom, J. C., Pooley, K. A., Klijn, J. G., Tollenaar, R. A., Devilee, P., & van Asperen, C. J. (2007). Clinical correlates of low-risk variants in FGFR2, TNRC9, MAP3K1, LSP1 and 8q24 in a Dutch cohort of incident breast cancer cases. Breast Cancer Res, 9(6), R78. https://doi.org/10.1186/bcr1793

Hunter, D. J., Kraft, P., Jacobs, K. B., Cox, D. G., Yeager, M., Hankinson, S. E., Wacholder, S., Wang, Z., Welch, R., Hutchinson, A., Wang, J., Yu, K., Chatterjee, N., Orr, N., Willett, W. C., Colditz, G. A., Ziegler, R. G., Berg, C. D., Buys, S. S., McCarty, C. A., Feigelson, H. S., Calle, E. E., Thun, M. J., Hayes, R. B., Tucker, M., Gerhard, D. S., Fraumeni, J. F., Jr., Hoover, R. N., Thomas, G., & Chanock, S. J. (2007). A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet, 39(7), 870-874. https://doi.org/10.1038/ng2075

Ibrahim, A., Khaled, H. M., Mikhail, N., Baraka, H., & Kamel, H. (2014). Cancer Incidence in Egypt: Results of the National Population-Based Cancer Registry Program. *Journal of cancer epidemiology*, *2014*, 437971. https://doi.org/10.1155/2014/437971

Jahan, N., Begum, M., Barek, M. A., Aziz, M. A., Hossen, M. S., Bhowmik, K. K., Akter, T., Islam, M. R., Abdulabbas, H. S., & Islam, M. S. (2023). Evaluation of the Association between FGFR2 Gene Polymorphisms and Breast Cancer Risk in the Bangladeshi Population. Genes, 14(4), 819. https://www.mdpi.com/2073-4425/14/4/819

Jara, L., Gonzalez-Hormazabal, P., Cerceño, K., Di Capua, G. A., Reyes, J. M., Blanco, R., Bravo, T., Peralta, O., Gomez, F., Waugh, E., Margarit, S., Ibañez, G., Romero, C., Pakomio, J., & Roizen, G. (2013). Genetic variants in FGFR2 and MAP3K1 are associated with the risk of familial and early-onset breast cancer in a South-American population. Breast Cancer Res Treat, 137(2), 559-569. https://doi.org/10.1007/s10549-012-2359-z

Kamińska, M., Ciszewski, T., Łopacka-Szatan, K., Miotła, P., & Starosławska, E. (2015). Breast cancer risk factors. Prz Menopauzalny, 14(3), 196-202. https://doi.org/10.5114/pm.2015.54346

Kandil, N. S., Kandil, L. S., Mohamed, R., Selima, M., El Nemr, M., Barakat, A. R., & Alwany, Y. N. (2022). The Role of miRNA-182 and FOXO3 Expression in Breast Cancer. *Asian Pac J Cancer Prev*, *23*(10), 3361-3370. https://doi.org/10.31557/apjcp.2022.23.10.3361

Ledwoń, J. K., Hennig, E. E., Maryan, N., Goryca, K., Nowakowska, D., Niwińska, A., & Ostrowski, J. (2013). Common low-penetrance risk variants associated with breast cancer in Polish women. *BMC Cancer*, *13*, 510. https://doi.org/10.1186/1471-2407-13-510

Liang, J., Chen, P., Hu, Z., Zhou, X., Chen, L., Li, M., Wang, Y., Tang, J., Wang, H., & Shen, H. (2008). Genetic variants in fibroblast growth factor receptor 2 (FGFR2) contribute to susceptibility of breast cancer in Chinese women. Carcinogenesis, 29(12), 2341-2346. https://doi.org/10.1093/carcin/bgn235

Lin, C. Y., Ho, C. M., Bau, D. T., Yang, S. F., Liu, S. H., Lin, P. H., Lin, T. H., Tien, N., Shih, M. C., & Lu, J. J. (2012). Evaluation of breast cancer susceptibility loci on 2q35, 3p24, 17q23 and FGFR2 genes in Taiwanese women with breast cancer. *Anticancer Res*, *32*(2), 475-482.

Mahdavi, M., Nassiri, M., Kooshyar, M. M., Vakili-Azghandi, M., Avan, A., Sandry, R., Pillai, S., Lam, A. K., & Gopalan, V. (2019). Hereditary breast cancer; Genetic penetrance and current status with BRCA. J Cell Physiol, 234(5), 5741-5750. https://doi.org/10.1002 /jcp.27464

Mahdi, K. M., Nassiri, M. R., & Nasiri, K. (2013). Hereditary genes and SNPs associated with breast cancer. *Asian Pac J Cancer Prev*, *14*(6), 3403-3409. https://doi.org/10.7314/apjcp.2013.14.6.3403

Marian, C., Ochs-Balcom, H. M., Nie, J., Kallakury, B. V., Ambrosone, C. B., Trevisan, M., Edge, S., Shields, P. G., & Freudenheim, J. L. (2011). FGFR2 intronic SNPs and breast cancer risk: associations with tumor characteristics and interactions with exogenous exposures and other known breast cancer risk factors. Int J Cancer, 129(3), 702-712. https://doi.org/10.1002/ijc.25686

McInerney, N., Colleran, G., Rowan, A., Walther, A., Barclay, E., Spain, S., Jones, A. M., Tuohy, S., Curran, C., Miller, N., Kerin, M., Tomlinson, I., & Sawyer, E. (2009). Low penetrance breast cancer predisposition SNPs are site specific. Breast Cancer Res Treat, 117(1), 151-159. https://doi.org/10.1007/s10549-008-0235-7

Mehrgou, A., & Akouchekian, M. (2016). The importance of BRCA1 and BRCA2 genes mutations in breast cancer development. Med J Islam Repub Iran, 30, 369.

Metcalfe, K., Lubinski, J., Lynch, H. T., Ghadirian, P., Foulkes, W. D., Kim-Sing, C., Neuhausen, S., Tung, N., Rosen, B., Gronwald, J., Ainsworth, P., Sweet, K., Eisen, A., Sun, P., & Narod, S. A. (2010). Family history of cancer and cancer risks in women with BRCA1 or BRCA2 mutations. *J Natl Cancer Inst*, *102*(24), 1874-1878. https://doi.org/10.1093/jnci/djq443

Meyer, K. B., Maia, A. T., O'Reilly, M., Teschendorff, A. E., Chin, S. F., Caldas, C., & Ponder, B. A. (2008). Allele-specific up-regulation of FGFR2 increases susceptibility to breast cancer. PLoS Biol, 6(5), e108. https://doi.org/10.1371/journal.pbio.0060108

Moffa, A., Tannheimer, S., & Ethier, S. (2004). Transforming Potential of Alternatively Spliced Variants of Fibroblast Growth Factor Receptor 2 in Human Mammary Epithelial Cells. Molecular cancer research : MCR, 2, 643-652. https://doi.org/10.1158/1541-7786.643.2.11

Neamatzadeh, H., Shiryazdi, S. M., & Kalantar, S. M. (2015). BRCA1 and BRCA2 mutations in Iranian breast cancer patients: A systematic review. J Res Med Sci, 20(3), 284-293.

Nounou, M. I., ElAmrawy, F., Ahmed, N., Abdelraouf, K., Goda, S., & Syed-Sha-Qhattal, H. (2015). Breast Cancer: Conventional Diagnosis and Treatment Modalities and Recent Patents and Technologies. Breast Cancer (Auckl), 9(Suppl 2), 17-34. https://doi.org/10.4137 /bcbcr.S29420

Ozgöz, A., Samli, H., Oztürk, K. H., Orhan, B., Icduygu, F. M., Aktepe, F., & Imirzalioglu, N. (2013). An investigation of the effects of FGFR2 and B7-H4 polymorphisms in breast cancer. J Cancer Res Ther, 9(3), 370-375. https://doi.org/10.4103/0973-1482.114434

Pal, M., Das, D., & Pandey, M. (2024). Understanding genetic variations associated with familial breast cancer. *World Journal of Surgical Oncology*, *22*(1), 271. https://doi.org/10.1186/s12957-024-03553-9

Qiu, M., Olsen, A., Faivre, E., Horwitz, K. B., & Lange, C. A. (2003). Mitogen-activated protein kinase regulates nuclear association of human progesterone receptors. Mol Endocrinol, 17(4), 628-642. https://doi.org/10.1210/ me.2002-0378

Rebbeck, T. R., DeMichele, A., Tran, T. V., Panossian, S., Bunin, G. R., Troxel, A. B., & Strom, B. L. (2009). Hormone-dependent effects of FGFR2 and MAP3K1 in breast cancer susceptibility in a population-based sample of post-menopausal African-American and European-American women. *Carcinogenesis*, *30*(2), 269-274. https://doi.org/10.1093/carcin/bgn247

Sacks DB. Diabetes Mellitus. In: Burtis CA, Ashwood ER, Bruns DE,(eds). Tietz textbook of clinical chemistry and molecular diagnostics. 5th ed. Missouri, St Louis: Elsevier Saunders Co; 2012. p.718-21.

Shen, L., Zhang, S., Wang, K., & Wang, X. (2021). Familial Breast Cancer: Disease Related Gene Mutations and Screening Strategies for Chinese Population. *Front Oncol*, *11*, 740227. https://doi.org/10.3389/fonc.2021 .740227

Shu, J., Hui, X., Zheng, X., Zhao, J., Xu, Z., Chen, Y., Lu, C., & Li, J. (2019). Correlation of FGFR2 rs2981582 polymorphisms with susceptibility to breast cancer: a case-control study in a Chinese population. J Int Med Res, 47(10), 4753-4763. https://doi.org/10.1177/ 0300060519869058.

Siddiqui, S., Chattopadhyay, S., Akhtar, M. S., Najm, M. Z., Deo, S. V., Shukla, N. K., & Husain, S. A. (2014). A study on genetic variants of Fibroblast growth factor receptor 2 (FGFR2) and the risk of breast cancer from North India. *PLoS One*, *9*(10), e110426. https://doi.org/10.1371/journal.pone.0110426

Slattery, M. L., Baumgartner, K. B., Giuliano, A. R., Byers, T., Herrick, J. S., & Wolff, R. K. (2011). Replication of five GWAS-identified loci and breast cancer risk among Hispanic and non-Hispanic white women living in the Southwestern United States. Breast Cancer Res Treat, 129(2), 531-539. https://doi.org/ 10.1007/s10549-011-1498-y

Sumbal, J., & Koledova, Z. (2019). FGF signaling in mammary gland fibroblasts regulates multiple fibroblast functions and mammary epithelial morphogenesis. *Development*, *146*(23). https://doi.org/10.1242 /dev.185306

Sun, C., Olopade, O. I., & Di Rienzo, A. (2010). rs2981582 is associated with FGFR2 expression in normal breast. *Cancer Genet Cytogenet*, *197*(2), 193-194. https://doi.org/10.1016/j.cancergencyto.2009.11.006

Sun, S., Jiang, Y., Zhang, G., Song, H., Zhang, X., Zhang, Y., Liang, X., Sun, Q., & Pang, D. (2012). Increased expression of fibroblastic growth factor receptor 2 is correlated with poor prognosis in patients with breast cancer. J Surg Oncol, 105(8), 773-779. https://doi.org/10.1002/jso.22120

Tamaru, N., Hishikawa, Y., Ejima, K., Nagasue, N., Inoue, S., Muramatsu, M., Hayashi, T., & Koji, T. (2004). Estrogen receptor-associated expression of keratinocyte growth factor and its possible role in the inhibition of apoptosis in human breast cancer. Lab Invest, 84(11), 1460-1471. https://doi.org/10.1038/labinvest.3700166

Thompson, D., & Easton, D. (2004). The genetic epidemiology of breast cancer genes. *J Mammary Gland Biol Neoplasia*, *9*(3), 221-236. https://doi.org/10.1023/ B:JOMG.0000048770.90334.3b

Tozlu, S., Girault, I., Vacher, S., Vendrell, J., Andrieu, C., Spyratos, F., Cohen, P., Lidereau, R., & Bieche, I. (2006). Identification of novel genes that co-cluster with estrogen receptor alpha in breast tumor biopsy specimens, using a large-scale real-time reverse transcription-PCR approach. Endocr Relat Cancer, 13(4), 1109-1120. https://doi.org/ 10.1677/erc.1.01120

Turnbull, C., & Rahman, N. (2008). Genetic predisposition to breast cancer: past, present, and future. Annu Rev Genomics Hum Genet, 9, 321-345. https://doi.org/10.1146/annurev.genom.9.081307.164339

Wesche, J., Haglund, K., & Haugsten, E. M. (2011). Fibroblast growth factors and their receptors in cancer. Biochem J, 437(2), 199-213. https://doi.org/10.1042 /bj20101603

Yang, J. Y., & Hung, M. C. (2009). A new fork for clinical application: targeting forkhead transcription factors in cancer. Clin Cancer Res, 15(3), 752-757. https://doi.org/10.1158/1078-0432.Ccr-08-0124

Yasam, S. K., Chandrashekar, G., Ganapathy, P., Jaganathan, R., Kulanthaivel, L., & Subbaraj, G. K. (2023). Association of FGFR2 rs2981582 polymorphism and breast cancer susceptibility: An updated systematic review and meta-analysis. *Human Gene*, *37*, 201212. https://doi.org /https://doi.org/10.1016/j.humgen.2023.201212 .