RESEARCH ARTICLE

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Genetic Polymorphism in *FSCN1 rs3801004 C/G* and *CD44 rs353639 A/C*, as Prognostic Factor in Egyptian Breast Cancer Patients

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Abstract

Background: One of the main causes of cancer-related deaths is breast cancer. Fascin-1(FSCN1) is an actin-binding protein that is present in the mesenchymal, neuronal, and endothelial cells of mammals. Patients with breast cancer have been found to have *FSCN1* overexpression. *CD44* is crucial for the development, invasion, and tumour spread. Therefore, we aimed to investigate the role of *FSCN1&CD44* gene polymorphisms in breast cancer (BC) risk and prognosis. **Materials & Methods:** A total of 96 BC patients and 50 controls were included in the case-control study for risk prediction. We examined the association between The SNPs on *FSCN1(rs3801004)* and *CD44(rs353639)* and BC susceptibility and clinicopathological features using a real-time PCR in a cohort of the Egyptian population. **Results:** A significant association of both SNPs on *FSCN1(rs3801004)*C allele and *CD44(rs353639)*A allele and BC susceptibility(adjusted OR=4.38,95%CI:2.6–7.4,p<0.001, and adjusted OR=4.44,95%CI:2.65–7.44,p <0.001,respectively). Moreover, CC genotype in *FSCN1(rs3801004)* were likely to progress to developing G2&G3 and N2&N3 and stage II & stage IV, according to the TNM staging and GG+GC genotypes increased within individuals who had a positive family history of BC. Individuals who carry at least one A allele for *CD44rs353639* were likely to progress developing N2 according to the TNM in BC patients. **Conclusions:** These findings suggest that both SNPs on *FSCN1 (rs3801004)* and *CD44 (rs353639)* affected BC susceptibility. *FSCN1 (rs3801004)* genetic variants may have a significant effect on BC prognosis. However, *CD44 (rs353639)* affected lymph node invasions in BC patients.

Keywords: Breast cancer- Fascin-1(*FSCN1*)- *CD44*- gene polymorphisms

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Introduction

One of the most prevalent malignancies that contribute to the mortality of women globally is breast cancer (BC). Every year, more than a million women are diagnosed with breast cancer worldwide, and an additional 400,000 are reported to pass away from the condition (Jemal et al., 2011). Although BC is a worldwide problem, the number of cases in Egypt is concerning. BC is thought to be the most prevalent cancer in women, accounting for 32.04% of cases between the years 2008 and 2011, according to data from the National Cancer Registry Program of Egypt (NCRPE). Additionally, it accounts for 29.1% of all cancer-related deaths (Zeeneldin et al.,2013; Ibrahim et al., 2014) . Breast cancer development and prognosis are known to be significantly influenced by environmental and genetic variables. Finding these factors can help to lower the incidence of breast cancer because they can act as a susceptibility factor for the development of breast cancer (Omran et al., 2021).

Breast cancer-initiating cells (BCICs), a small subset of unique cells found in breast malignancies, are identified by the expression of cancer-initiating cells (CIC) biomarkers (Lobo et al., 2007). Among them is the biomarker *CD44*. Chromosome 11p13 contains the *CD44* gene (Goodfellow et al., 1982). The protein that is encoded is a cell surface glycoprotein that has a role in a variety of biological functions, including lymphocyte mobility, extravasation, homing, activation, and apoptosis (Bourguignon et al., 1998 ; Sales et al., 2007). Additionally, numerous studies have noted its part in tumour metastasis (Marhaba and Zoller, 2004; Hill et al., 2006). It serves as a hyaluronic

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acid receptor as well. According to much research, *CD44* regulates the proliferation, migration, and invasion of breast cancer cells through its interaction with hyaluronan. Additionally, it was discovered that *CD44* genetic variations were linked to breast cancer patient prognosis, risk assessment, and survival (Jiang et al., 2012; Xin and Cheng-yi, 2012). According to certain studies, the SNPs rs13347 and rs353639 were significantly linked to breast cancer risk and prognosis in North Indian and Chinese populations, respectively (Jiang et al., 2012; Xin and Cheng-yi, 2012; Zhou et al., 2010).

Fascin-1 (*FSCN1*), an actin-binding protein present in mammalian cells, is only minimally or completely absent in normal epithelial cells such as endothelial, neuronal, and mesenchymal cells (Kureishy et al., 2002). *FSCN1* has been shown to be upregulated in a variety of cancer cell types, including those found in the stomach, colon, lung, ovary, and breast (Zhao et al., 2010; Jawhari et al., 2003; Yoder et al., 2005). Significantly increasing colon cancer cell migration and metastasis is made possible by overexpressing *FSCN1* (Vignjevic et al., 2007), whereas *FSCN1* knockdown in cellular models reduces prostate cancer cell motility and tumour spread (Darnel et al., 2009) and oral squamous cancer (Chen et al., 2009). The signal transducer and activator of the transcription 3 (STAT3) signaling pathway are activated by a number of cytokines, such as interleukin-6 and oncostatin M, which regulate fascin expression in breast cancer cells (Snyder et al., 2011). Recent research indicates that abnormal STAT3 signaling accelerates the development of breast tumours by downregulating the expression of downstream target genes that regulate angiogenesis, such as nuclear factor kappaB (NF-kappaB) and hypoxia-inducible factor 1 (HIF1), and by increasing their binding to the fascin gene promoter to activate its expression (Yao et al., 2014) . Attractively, in African American women with triple-negative breast cancer, a highly significant connection has been found between fascin expression and a reduced overall survival rate (TNBC) (Esnakula et al., 2014) . In a similar vein, they have previously discussed how very positive *FSCN1* expression can be utilized as a diagnostic indicator of TNBC in Chinese women (Wang et al., 2016). It is yet unclear whether there is a connection between *FSCN1* SNPs and breast cancer risk or prognosis. In order to assess the significance of the SNPs on *FSCN1 (rs3801004)* and CD44 (rs353639) in breast cancer susceptibility and clinicopathological characteristics in a cohort of Egyptian individuals, we thus carried out a case-control study.

Materials and Methods

Participants

The current study used 96 blood samples from breast cancer patients who had received a clinical diagnosis of the disease and had it confirmed through mammography and surgical biopsies at the General Surgery Department of the Faculty of Medicine, Cairo University. The ages of the patients ranged from 28 to 66, with a mean of 46 + 9.8 years. Prior to enrolling in the trial, none of the patients had received radiation, chemotherapy, or anti-hormonal medication. Fifty healthy female volunteers of similar age were also enrolled as the "normal control group." Healthy patients admitted to the same hospital served as their source of recruitment. None of them had liver, kidney, or hypertension issues. In addition to not using birth control, they had no palpable breast lumps. A histopathological examination verified the cancer diagnosis. Clinical data from the hospital records, including stage, grade, hormone receptor status (ER, PR, and Her2), tumour size, and clinical lymph node, were retrieved. For the examination of genetic differences, all clinicopathological information was gathered from medical records (Table 1). All measures were carried out following the ethical considerations of the Research Committee of Kasr Al-Ainy, Cairo University Hospitals, as well as the ethical standards of the 1964 Declaration of Helsinki (approval number). Prior to data collection and after being informed of the study's goals, all participants gave their informed consent.

Extraction of peripheral blood DNA

Whole blood samples were collected and whole genomic DNA was extracted using (Qiagen, Milan Italy), as directed by the manufacturer. The Tris-EDTA (TE) buffer, which is made of 10 mM Tris-HCl and 1 mM EDTA•Na2, was used to dissolve the DNA (pH 7.8). Prior to being used in a quantitative polymerase chain reaction (PCR) assay, Prior to quantitative polymerase chain reaction (PCR) analysis, pure genomic DNA samples were measured using ultraviolet absorbance at 260 nm using a Thermo Scientific NanoDrop TM and kept at 20 °C.

Genotyping of the FSCN1 rs3801004 C/G and CD rs353639 A/C polymorphisms

Using a real-time PCR methodology based on the prevalidated TaqMan MGBTM probe for allelic discrimination test, the SNPs on *FSCN1 (rs3801004)* and *CD44 (rs353639)* were found (Applied Biosystems). In a final volume of 25 mL of DNAse/RNAse-free water (Invitrogen/Life Technologies, USA) and template, 1.25 mL of a 40X combined primer and probe mix (ABI/ Life Technologies, USA) was added to 12.5 mL of a 2X TaqManTM Universal PCR master mix (ABI/Life Technologies, USA). 95 °C for 10 min, 95 °C for 15 s, and 60 °C for 1 min were the cycle conditions. 40 repetitions total were given to the final two phases. The Rotor-Gene real-time PCR system was used to carry out the PCR run (Qiagen, Santa Clarita, CA). The Statistical Package for the Social Sciences (SPSS version 16.0; SPSS, Chicago, IL) program was used to create allelic discrimination plots.

Statistical analysis

SPSS (Statistical Package for the Social Sciences) version 22 was used for statistical analysis. In the case of quantitative variables, statistics are expressed as means S.E., and in the case of categorical variables, as frequencies and percentages. The odds ratios (ORs) of breast cancer associated with each genotype with 95% confidence intervals (CI) were estimated by logistic regression analysis. Independent Student t-tests were used to compare categorical data and quantitative factors. To evaluate the OR connecting various genotypes with

clinic pathological traits, logistic regression studies were also carried out. Statistical significance was defined as a p-value equal to or less than 0.05 (Chan, 2004).

Results

Characteristics of participants

Table 1 provides an overview of the case and control groups' demographic and clinico-pathological traits. Regarding age distribution, there was no significant difference between the case and control groups.

In light of the clinicopathological information, (54.2%) of all patients presented with (Grade 2) and 52 (36.5%) with (grade 3), while only nine patients (9.4%) were diagnosed with (Grade 1). For the TNM staging, the percentage of patients with T 2,3 and 4 were 64.4%, 28.1%, and 7.3%, respectively, N1, 2, and 3 were present in 14.6 percent, 51 percent, and 34.4 percent of patients, respectively. In terms of the estrogen receptors (ER), 65 patients (67.7%) tested positive, and 56 patients (58.3%) tested positive for the progesterone receptors (PR). Finally, 43(44.8%) of the patients were positive for HER2 neu protein. Tumors were graded using the modified Bloom-Richardson system, which included the following criteria: T size of the tumour, T1 (less than 2 cm), T2 (between 2 and 5 cm), T3 (above 5 cm), T4 (infiltration of the skin and chest wall), N involvement of local lymph nodes, ER (estrogen receptor), PR (progesterone receptor), and T (less than or equal to 2 cm). N1 cancer has progressed to one to three axillary lymph nodes and/or lymph node biopsy results have revealed modest quantities of malignancy in internal mammary lymph nodes. N2 carcinoma has grown the internal mammary lymph nodes or has migrated to 4 to 9 axillary lymph nodes. N3 carcinoma has progressed to the internal mammary lymph nodes, the axillary lymph nodes, or both. (Table 1)

Table 1. Demographic, Laboratory, and Clinical Data in Control and Breast Cancer Groups

Variables	Controls N=200 (%)	Patients N=384 (%)	p-value
Age	Mean ± S.D.	Mean ± S.D.	0.8
	40.7±10.13	46±9.8	
Menopausal status			
Pre	19 (38%)	36 (37.5%)	0.95
Post	31 (62%)	60 (62.5%)	
Family History			
Negative	……	72 (75%)	……
Positive		24 (25%)	
Tumor size			
T1			
T2	……	62 (64.6%)	……
T3		27 (28.1%)	
T4		7 (7.3%)	
Grade			
G1	……	9 (9.4%)	……
G2		52 (54.2%)	
G3		35 (36.5%)	
Stage			
I	……	12 (12.5%)	……
II		52 (54.2%)	
III		24 (25%)	
IV		8 (8.3%)	
Lymph nodes invasion			
N1	……	14 (14.6%)	……
N2		49 (51%)	
N3		33 (34.4%)	
Metastasis			
M0	……	88 (91.7%)	……
M1		8 (8.3%)	
ER			
Negative	……	31 (32.3%)	……
Positive		65 (67.7%)	
PR			
Negative	……	40 (40.7%)	……
Positive		56 (58.3%)	
HER2 neu			
Negative	……	43 (44.8%)	……
Positive		53 (55.2%)	

Frequency Distribution of BC Patients' Alleles and Genotypes Compared to Control Subjects.

Table 2 displays the distribution of the various genotypes and alleles of *FSCN1 (rs3801004)* and *CD44 (rs353639)*. When we compared the healthy group to the BC patients, we discovered that the *FSCN1 (rs3801004)* CC genotype compared to CG and GG genotypes and the C allele compared to the G allele were linked strongly to a high incidence of breast cancer respectively (adjusted OR = 0.133, 95% CI:0.0537–0.3297, p <0.0001, adjusted OR = 0.1025, 95% CI: 0.0370–0.2834, p =<0.0001 and adjusted OR = 4.38, 95% CI: 2.6–7.4, p <0.001, respectively). However, the GG genotype and G allele was notably linked to a reduced incidence of BC (adjusted OR = 0.1025, 95% CI: 0.0370–0.2834, p <

Table 2. Genotype and Allele Frequencies of the FSCN1 rs3801004 C/G and CD44rs353639 A/C Polymorphisms in Breast Cancer Patients and Healthy Controls

	Control	Breast cancer	OR (95 % CI)
FSCN1 (rs3801004)			
C more than G			
CC	9 (18%)	62 (64.6%)	
CG	24 (48%)	22 (22.9%	0.133 (0.0537± 0.3297)
GG	17 (34%)	12 (12.5%)	0.1025 (0.0370 ± 0.2834)
C allele	42 (42%)	146 (76%)	4.38 (2.6 ± 7.4)
G allele	58 (58%)	46 (24%)	
P_{HW}*	0.9168	<0.001	
CD44(rs353639) Amore than c			
AA	6 (12%)	59 (61.5%)	
AC	29 (58%)	27 (28.1%)	0.0947 (0.0352 ± 0.2548)
CC	15 (30%)	10 (10.4%)	0.0678 (0.0213 ± 0.2163)
A allele	41 (41%)	145 (75.5%)	
C allele	59 (59%)	47 (24.5%)	4.44 (2.65 ± 7.44)
P_{HW}*	0.1597	0.019	

Table 3. Relationship between Clinicopathological Variables in BC Patients and the FSCN1 (rs3801004) Polymorphism

Clinicopathological parameters	CC	CG+GG	OR (95%CI)
menopausal status			
Post	23 (37.1%)	11 (32.4%)	1.233 (0.509-2.985)
Pre	39 (62.9%)	23 (67.6%)	
Family History			
Negative	51 (82.3%)	21 (61.8%)	2.87 (1.11-7.42)
Positive	11 (17.7%)	13 (38.2)	
Tumor size			
T2	38 (61.3%)	24 (70.6%)	
T3	18 (29%)	9 (26.5%)	0.792 (0.306-2.046)
T4	6 (9.7%)	1 (2.9%)	0.2639 (0.0299-2.3294)
Grade			
G1	2 (3.2%)	7 (20.6%)	
G2	35 (56.5%)	17 (50%)	0.1388 (0.026-0.7408)
G3	25 (40.3%)	10 (29.4%)	0.1143 (0.0202-0.674)
Stage			
I	3 (4.8%)	9 (26.5%)	
II	37 (59.7%)	15 (44.1%)	0.1351 (0.0321-0.569)
III	15 (24.2%)	9 (26.5%)	0.2 (0.0426-0.939)
IV	7 (11.3%)	1 (2.9%)	0.0476 (0.004-0.5626)
Lymph nodes invasion			
N1	4 (6.5%)	10 (29.4%)	
N2	35 (56.5%)	14 (41.2%)	0.16 (0.043-0.596)
N3	23 (37.1%)	10 (29.4%)	0.174 (0.0439-0.689)
Metastasis			
M0	55 (88.7%)	33 (97.1%)	0.238 (0.028-2.0223)
M1	7 (11.3%)	1 (2.9%)	
ER			
Negative	23 (37.1%)	8 (23.5%)	0.522 (0.203-1.343)
Positive	39 (62.9%)	26 (67.5%)	
PR			
Negative	23 (37.1%)	17 (50%)	1.6957 (0.727-3.955)
Positive	39 (62.9%)	17 (50%)	
HER2 neu			
Negative	25 (40.3%)	18 (52.9%)	1.665 (0.717-3.869)
Positive	37 (59.7%)	16 (47.1%)	

Table 4. Relation between the CD44 rs353639 A/C Polymorphism and Clinicopathological Parameters in BC Patients

Clinicopathological parameters	AA	AC+CC	OR (95%CI)
Menopausal status			
Post	23 (39%)	13 (35.1%)	0.8478 (0.361-1.99)
Pre	36 (61%)	24 (64.9%)	
Family History			
Negative	46 (78%)	26 (70.3%)	0.668 (0.262-1.703)
Positive	13 (22%)	11 (29.7%)	
Tumor size			
T2	36 (61%)	26 (70.3%)	
T3	19 (32.2%)	8 (21.6%)	1.72 (0.652-4.515)
T4	4 (6.8%)	3 (8.1%)	0.963 (0.198-4.673)
Grade			
G1	4 (6.8%)	5 (13.5%)	
G2	32 (54.2%)	20 (54.1%)	2.0 (0.479-8.346)
G3	23 (39%)	12 (32.4%)	2.396 (0.541-10.615)
Stage			
I	4 (6.8%)	8 (21.6%)	
II	33 (55.9%)	19 (51.4%)	3.473 (0.922-13.09)
III	16 (27.1%)	8 (21.6%)	4.0 (0.92-17.397)
IV	6 (10.2%)	2 (5.4%)	6.0 (0.812-44.353)
Lymph nodes invasion			
N1	5 (8.5%)	9 (24.3%)	
N2	32 (54.2%)	17 (45.9%)	3.39 (0.979-11.725)
N3	22 (37.3%)	11 (29.7%)	3.6 (0.97-13.357)
Metastasis			
M0	53 (89.8%)	35 (94.6%)	1.98 (0.378-10.38)
M1	6 (10.2%)	2 (5.4%)	
ER			
Negative	22 (37.3%)	9 (24.3%)	1.85 (0.739-4.632)
Positive	37 (62.7%)	28 (75.7%)	
PR			
Negative	22 (37.3%)	18 (48.6%)	0.6 (0.273-1.444)
Positive	37 (62.7%)	19 (51.4%)	
HER2 neu			
Negative	24 (40.7%)	19 (51.4%)	0.65 (0.284-1.487)
Positive	35 (59.3%)	18 (48.6%)	

0.001). And we revealed that the *CD44 (rs353639)* AA genotype compared to AC and CC genotypes and the A allele compared to the C allele were linked strongly to a high incidence of breast cancer respectively (adjusted OR = 0.0947, 95% CI:0.0352–0.2548, p<0.0001 and adjusted OR = 0.0678, 95% CI: 0.0213–0.2163, p<0.0001 and adjusted OR = 4.44, 95% CI: 2.65–7.44, p <0.001, respectively). However, the CC genotype and C allele were notably linked to a reduced incidence of BC (adjusted OR = 0.0678, 95% CI: 0.0213–0.216, p < 0.001). (Table 2).

Associations between clinicopathological Features of Patients with BC and the FSCN1 (rs3801004) Genotypes CC and (CG+GG)

We evaluated the relations between the *FSCN1 (rs3801004)* Genotypes CC, Genotypes (CG+GG), and clinicopathological Features of Patients with BC (Table 3)

When compared to people without a positive family history of BC, the incidence of the GG and GC genotypes was higher (adjusted OR = 2.87, 95 percent CI: 1.11-7.42, p = 0.0297). Regarding the Grade staging, BC patients with the CC genotype were more susceptible to developing G2 and G3 than those with the GG + GC genotype with respect to G1, respectively (adjusted OR =0.1388, 95% CI: 0.026–0.7408, p =0.020 and adjusted OR = 0.1143, 95% CI: 0.0202–0.674, p = 0.0142, respectively).

The frequency of the CC genotype was significantly associated with developing N2 and N3 of TNM staging with respect to N1 when compared to the GG+CG genotypes (adjusted OR = 0.16, 95 percent CI: 0.043-0.596, p = 0.006 and adjusted OR = 0.174, 95 percent CI: 0.0439-0.689, p = 0.0128, respectively).

According to the TNM staging, it is interesting to note

that BC patients with the CC genotype had a higher risk of developing stages II and IV compared to stages I (adjusted OR = 0.135, 95 percent CI: 0.0321-0.569, p = 0.006 and adjusted OR = 0.0476, 95 percent CI: 0.004-0.5626, p = 0.0157, respectively). But BC patients carrying the GG+CG genotypes were more susceptible to developing stage III (adjusted OR = 0.2, 95% CI: 0.0426–0.939, p = 0.041) (Table 3)

Associations between the CD44 rs353639 Genotypes AA, Genotypes (AC+CC) and ClinicoPathological Features of Patients with BC

We assessed the relations between the *CD44 rs353639* Genotypes AA, Genotypes (AC+CC), and clinicopathological Features of Patients with BC (Table 4).

Compared with the AC+CC genotypes, the frequency of the AA genotype was related to developing N2 and nearly too significant to developing N3 of TNM staging with respect to N1 (adjusted OR = 3.39, 95% CI: 0.979–11.725, p = 0.05 and adjusted OR = 3.6,95% CI: 0.97–13.357, p = 0.055, respectively) (Table 4).

Discussion

This study examines the Genetic Polymorphism in *FSCN1 rs3801004 C/G* as a Prognostic Biomarker in the Egyptian population of breast cancer patients. Moreover, we looked the relations between these *FSCN1 rs3801004* C/G SNPs and clinical status, clinical pathologic markers, and breast cancer susceptibility. In analyses that modified for potential confounding variables, the CC genotype of *FSCN1 (rs3801004)* compared to the CG and GG genotypes and the C allele compared to the G allele were substantially associated with a high incidence of breast cancer, respectively. (Adjusted OR=0.133, 95% CI: 0.0537–0.3297, p <0.0001, adjusted OR=0.1025, 95% CI: 0.0370–0.2834, p =<0.0001 and adjusted OR=4.38, 95% CI: 2.6–7.4, p <0.001, respectively). Moreover, CC genotype in *FSCN1(rs3801004)* were likely to progress developing G2&G3 and N2&N3 and stage II & stage IV, according to the TNM staging and GG+GC genotypes increased within individuals who had a positive family history of BC.

In line with our results, Wang et al., (2017) discovered that individuals who carried a minimum of one G allele in the rs3801004 locus were more likely to develop stage III/IV illness and lymph node metastases.

In patients with invasive ductal carcinoma (IDC), Liu and his colleagues found that *FSCN1* expression was related to a variety of poor prognostic factors. This finding raises the possibility that *FSCN1* may be connected to the development of breast carcinoma. They also confirmed the probable functional involvement of *FSCN1* expression in the development of TNBC because it was much higher in TNBC than in the non-TNBC subtype. As a result, their findings help to clarify the functional significance of *FSCN1* in the development of TNBC and could offer fresh insight into the mechanism underlying neoplastic progression. In addition to the positions mentioned below, *FSCN1* is anticipated to have additional functional responsibilities. For instance, a reduction in nodal signal transduction and endoderm development in *FSCN1* knockdown or deletion (Liu et al., 2016).

Transmembrane glycoprotein *CD44* is involved in a variety of processes, including metastasis, invasion, and cell growth (So et al., 2011). According to the findings, *CD44* plays a crucial role in the occurrence and prognosis of cancer. To assess the role of *CD44 (rs353639)* gene polymorphisms in Egyptian BC patients, the current study was conducted. The *CD44* gene's *SNP(rs353639)* was examined to investigate the relationship between genetic variations and the prognosis and risk of breast cancer. Few reports and limited research have been done on *CD44* gene polymorphisms in breast cancer globally (Jiang et al., 2012; Xin and Cheng-yi, 2012; Zhou et al., 2010; Gotte and Yip, 2006). In this study, A significant association of *CD44 (rs353639)* A allele and BC susceptibility (adjusted OR=4.44,95%CI:2.65–7.44, p<0.001,). Individuals who carry at least one A allele for *CD44rs353639* were likely to progress developing N2 according to the TNM in BC patients. Contrary to the results of the tour, research on the GIH population (Tulsyan et al., 2013).

Additionally, we observed a relationship between the frequency of the AA genotype and the development of N2 and a nearly significant relationship with the development of N3 of TNM staging with respect to N1 (adjusted OR = 3.39, 95 percent CI: 0.979-11.725, p = 0.05 and adjusted OR = 3.6, 95 percent CI: 0.97-13.357, p = 0.055, respectively).

According to Zhou et al. research, there is a specific SNP called *CD44* Ex2+14 A.G in the intron 1 area. Patients with this variant genotype had breast cancer at younger ages, bigger tumour burdens, additional local lymph nodes, and higher rates of recurrence of cancer (Zhou et al., 2010). The *CD44* polymorphisms in exon 2's coding sequence were found to be significantly correlated with greater probability and cumulative risk for breast cancer in a subsequent study by the same author that was published in 2012. Previous study sequenced exon 2 and detected 4 SNPs through it has been sequenced (Zhou et al., 2011). Additionally, a study revealed that in contrast to healthy breast has been detected epithelium, breast cancer significantly increased the expression of *CD44* (Bankfalvi et al., 1998). Therefore, in order to reach a firm conclusion, it is necessary to reproduce the preceding findings in a larger sample size of people of different ethnicities.

Our findings concur with the Tulsyans study on the relationship between the rs353639 polymorphism and breast cancer susceptibility (Tulsyan et al., 2013). Additionally, we did not find any associations when we performed sub-group analyses based on tumour size, grade, stage, metastasis, estrogen receptor (ER), progesterone receptor (PR), HER2 neu, and family history. This is in line with research done on the population of North Indians (Tulsyan et al., 2013).

However, there hasn't been any research on how the rs353639 polymorphism affects cancer risk in the Egyptian population. Because *CD44* polymorphisms play an indirect role in breast cancer susceptibility, there may be some variation in how these polymorphisms affect that susceptibility. And their effects may be mediated by connection to other important functional polymorphisms,

particularly in the exon 2 and exon 5 region of *CD44*'s hyaluronan binding. Therefore, it brought attention to the fact that genetic variations do not solely affect the development and prognosis of breast cancer. Along with these known variants, it is crucial to assess the influence of confounding factors such as age, clinical stage, diseased lymph node, grade, hormone, and Her 2 neu receptor. We did not detect a significant relationship between variant genotype (AC+CC) and C genotype and higher clinical tumour size or metastasis for SNP rs353639. Our findings are in opposition to the idea that genetic changes in the *CD44* gene may perhaps affect the altered binding of its ligand-hyaluronan-which results in higher breast cancer cell growth and differentiation. Our findings are in agreement with the study of Tulsyan and his colleagues. The significance of this finding was increased (Tulsyan et al., 2013).

In conclusion, our findings show a connection between breast cancer risk and *FSCN1* gene variations. We demonstrate that among Egyptian females, the *FSCN1 rs3801004* C/G polymorphisms significantly raise the risk of breast cancer progression. The association between *FSCN1* polymorphisms and breast cancer risk in the Egyptian population is first reported in this study. The predictive marker *FSCN1* may help determine the prognosis of breast cancer. Additionally, our findings suggested that the polymorphisms in the *CD44 rs353639* gene might affect the Egyptian population's ability to forecast breast cancer risk. Additionally, these polymorphisms have some effects on the prognosis of breast cancer. We believe that our study is the first to link an SNP of the *CD44* gene polymorphisms with breast cancer risk and prognosis in Egyptian participants.

Author Contribution Statement

Noha E. Ibrahim: participated in the construction of the hypothesis, performed the molecular techniques and biochemical analysis, and contributed to writing the manuscript and reviewed the manuscript, made significant revisions to the drafts and reviewing the article before submission.; Reham M. Raafat Hamed: performed the molecular techniques and biochemical analysis and contributed to writing and reviewed the manuscript, made significant revisions to the drafts and reviewing the article before submission and corresponding with the journal.; Ahmed Refaat: participated in the management of the patient and clinical examinations; Yasser O. Mosaad: contributed to reviewing the manuscript and contributed to doing statistics; Dina M. Mekawy: performed the molecular techniques and biochemical analysis and contributed to writing the manuscript and participated in the supervision of the course of this work. All authors have read and approved the final form of the manuscript.

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How the ethical issue was handled (name the ethical committee that approved the research)

All measures were carried out following the ethical considerations of the Research Committee of Kasr Al-Ainy, Cairo University Hospitals, as well as the ethical standards of the 1964 Declaration of Helsinki (approval number; N 39-2023). All subjects provided informed consent prior to data collection and following the explanation of research objectives.

Availability of data (if apply to your research)

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Any conflict of interest

All authors declare no conflict of interest.

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