Role of Irisin/FNDC5 (rs3480) Single Nucleotide Polymorphism in Breast Cancer

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Abstract:

Background: Breast cancer is the most common malignancy among women and remains a leading cause of cancer-related mortality. Previous studies suggest that the FNDC5/irisin gene may exert a protective role against breast cancer, as reduced expression levels have been linked to the presence of metastasis. The present study aims to investigate the potential association between single nucleotide polymorphisms (SNPs) in the irisin gene and the risk of breast cancer. In addition, it will analyze the distribution of these genetic alleles in relation to different clinical parameters of the disease

Patients & Methods: This cross-sectional study was conducted from July 2023 to May 2024 and included 50 pathologically confirmed breast cancer patients recruited from the Clinical Oncology Department, Faculty of Medicine, Sohag University. A control group of 50 healthy volunteers, with no clinical evidence of any neoplastic disorder, was also enrolled. Patient characteristics were retrieved from the local databases of the Clinical Oncology Department at Sohag University.

Results: Our study revealed no significant differences between cases and controls regarding age or family history. Circulating irisin levels showed a highly significant reduction in breast cancer patients compared to controls ($P < 0.001$). In addition, a statistically significant association was identified between genotypic distribution and tumor pathology ($P < 0.005$). The GG genotype was predominantly linked to invasive lobular carcinoma (33.3%), whereas the AA and AG genotypes were more frequently associated with invasive ductal carcinoma

Conclusion: The study concluded that circulating irisin appears to play a protective role against breast cancer, as its levels were markedly lower in patients compared to controls. While irisin gene polymorphism may not represent a direct risk factor for breast cancer, it could influence tumor behavior of breast cancer and disease severity.

Key words: Breast cancer, irisin gene, control group, genotypes

Received: 28 August 2025
Accepted: 30 September 2025

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Introduction:

Breast cancer is the most common malignancy among women and represents the second leading cause of cancer-related deaths [1]. FNDC5/irisin has been suggested to play a protective role against breast cancer, as higher levels of FNDC5/irisin have been associated with reduced metastatic potential and improved patient survival [2]. Irisin is a hormone cleaved from the FNDC5 protein, which is expressed in several tissues, particularly in skeletal muscle fibers, where its production increases in response to physical activity. This rise promotes irisin secretion, which subsequently regulates metabolism and enhances thermogenesis in adipose tissue [3, 4].

Structurally, irisin is generated through cleavage of a 112–amino acid sequence from FNDC5 [4]. The resulting protein undergoes glycosylation, a modification required for its secretion. The unglycosylated form has a molecular weight of approximately 13 kDa, while the glycosylated form is heavier, around 20 kDa. When glycosylation is inhibited, the secretion of irisin is markedly reduced [5]. The role of irisin in cancer remains debated. Some studies report reduced circulating levels in breast cancer

patients [6], whereas others observed increased expression in different malignancies, including breast, prostate, liver, and lung cancers [7,8].

Aim of the Study:

The present study aimed to investigate the potential association between irisin gene single nucleotide polymorphisms (SNPs) and breast cancer risk, as well as the distribution of its alleles in relation to various clinical parameters within the breast cancer cohort.

Patients and Methods:

This cross-sectional study was conducted between July 2023 and May 2024 and included 50 pathologically confirmed breast cancer patients and 50 healthy volunteers as controls. All participants were recruited from the Clinical Oncology Department, Faculty of Medicine, Sohag University. Patient characteristics were retrieved from the department's local database. The study protocol was approved by, Research Ethics Committee Faculty of Medicine Sohag University (IRB: Soh-Med-23-04-10PD), registered at ClinicalTrials.gov (NCT05876728), and written informed consent was obtained from all participants.

Data collection:

All enrolled patients underwent a comprehensive assessment that included a detailed medical history covering age, family history, marital and menopausal status. A full general and systemic examination was performed. Tumor-related characteristics were also documented, including tumor size, location, histopathological type and grade. Genotyping of FNDC5 (rs3480) and irisin assay were done.

FNDC5 (rs3480) genotying and irisin assay:

Venous blood samples (3 mL) were collected in EDTA tubes and after centrifugation DNA was extracted by the Qiagen DNA extraction kit (Cat. No. 51304). The extracted DNA was then used for genotyping of the FNDC5 gene (rs3480) by one-step real-time polymerase chain reaction (PCR). The primers were sourced from Applied BioSystem: rs3480 AGACCGGAAGGAAGGAA (F-primer), TGGTCCCC AAGGGGCGGTCATT(A/G)GGTGATGGCTTCTGG CTCTCTGGCT.

For genotyping the FNDC5 (rs3480) (A/G) polymorphism, the eluted DNA was stored at -20°C. An allelic discrimination assay with real-time PCR (TaqMan Master Mix, Applied Biosystems) was used for genotyping. The qPCR Master Mix (300x) context sequence was provided by Thermo Fisher Scientific (catalog no. 4351379). The context sequence for irisin rs3480 AGACCGGAAGGAAGGAA (F-primer), TGGTCCCC AAGGGGCGGTCATT(A/G)GGTGATGGCTTCTGG CTCTCTGGCT.

The PCR reaction mixture consisted of 10 µL of master mix, 1.25 µL of SNP genotyping assay (primer mix), and 3.75 µL of D Nase-free water. A total of 5 µL of genomic DNA extract from each sample was added, while 5 µL of DNase-free water served as the negative control. Thermal cycling conditions were as follows: initial denaturation at 95 °C for 10 minutes, followed by 40 cycles of denaturation at 95 °C for 15 seconds, primer annealing at 60 °C for 60 seconds, and extension at 72 °C for 2 minutes. A final extension step was performed at 72 °C for 1 minute. Data acquisition and analysis were performed using the StepOne™ Real-Time PCR System (Applied Biosystems, Singapore). Serum irisin levels were quantified using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Crystal Day Biotech Co., Shanghai, China). Measurements were carried out using Stat Fax analyzers (Stat Fax 2200, Stat Fax 2600, and Stat Fax 2100, Awareness Technology Inc., USA).

Statistical analysis:

The data were entered and analyzed using SPSS version 27 (Statistical Package for the Social Sciences). Results were summarized as frequencies, percentages, means, and standard deviations. The Chi-square test and Fisher's exact test were applied to assess associations between categorical variables, while the independent-samples t-test was used to compare continuous variables across groups. A p-value of less than 0.05 was considered statistically significant.

Results:

Table 1 in our study presents the baseline clinical characteristics of the two groups. No significant differences were observed between them regarding age, family history, or menopausal status ($p > 0.05$). The majority of participants in both groups were premenopausal, accounting for 58% of cases and 56% of controls.

Table 2 outlines the tumor characteristics in the breast cancer patient group. The findings indicate that the mean tumor size was 3.20 ± 1.08, with a median of 4.0 cm, and tumors were more frequently located on the right side (62%) compared to the left (38%). The majority of tumors were classified as grade II (78%), followed by grade III (16%) and grade I (6%). Invasive ductal carcinoma represented the predominant subtype (94%), while invasive lobular carcinoma accounted for only 6%. Additionally, 68% of patients presented with negative lymph nodes, whereas 32% showed lymph node involvement. Hormone receptor status was positive in most cases (76%) and negative in 24%. Regarding HER2 status, 56% were negative, 40% positive, and 4% were not assessed.

Table 3 demonstrates a highly significant difference in circulating irisin levels between the two groups ($p < 0.001$). The mean serum irisin concentration in the patient group was markedly lower (2.55 ± 0.59 µg/ml) compared with the control group (3.57 ± 1.60 µg/ml). This finding supports the hypothesis that irisin may exert a protective role against breast cancer.

Table 4 presents the distribution of the three genotypes as follows: AA was the most frequent, detected in 42.0% of cases and 52.0% of controls; AG was observed in 48.0% of cases and 40.0% of controls; and GG was the least common, identified in 10.0% of

cases and 8.0% of controls. Regarding allele frequencies, allele A was found at a rate of 66% in the case group and 72% in the control group, while allele G was detected in 34% of cases and 28% of controls. No statistically significant differences were observed in the distribution of genotypes (AA, AG, GG) or allele frequencies (A, G) between the two groups ($p = 0.6$ and $p = 0.3$, respectively). These findings suggest that the irisin/FNDC5 rs3480 polymorphism may not represent a risk factor for breast cancer in this cohort.

Table 5 also demonstrates a statistically significant association between genotype distribution and tumor subtype ($p < 0.005$). The GG genotype was strongly linked to invasive lobular carcinoma (33.3%), whereas the AA and AG genotypes were predominantly associated with invasive ductal carcinoma. A significant relationship was further observed between genotype and tumor grade ($p < 0.005$). Specifically, the GG genotype was mainly related to grade I tumors (60%), while AA and AG genotypes were more frequently connected with grades II and III. These findings suggest that genetic variation may influence both tumor behavior and aggressiveness.

Table (1): Socio-demographic and clinical characteristics of the studied populations

Variable		Cases N=50	Controls N=50	P value
Age	*Mean ± SD*	9.15 ± 44.9	5.8 ± 47.0	0.9
	Median(range)	45 (51_40)	45 (51_41)	
Family history	*Yes*	0 (0%)	0 (0%)	--
	No	50 (100%)	50 (100%)	
Marital status	*Married*	50 (100%)	50 (100%)	--
	Single	0(0%)	0(0%)	
Menopausal status	*Pre-menopausal*	29(58%)	28 (56%)	0.8
	Post menopausal	21 (42%)	22 (44%)	

*P value was calculated by Independent T test or Chi square test wherever suitable

Table (2) Tumor characteristics of the cases

Variable		Cases N=50
Tumor size	Mean ± SD	1.08± 3.20
	Median(range)	4.0 (2.0 – 6.0)
Tumor site	Left	19(38%)
	Right	31(62%)
Tumor grade	Grade I	3 (6.0%)
	Grade II	39 (78.0%)
	Grade III	8 (16.0%)
Pathological type	Invasive ductal	47(94%)
	Invasive lobular	3(6%)
Lymph node status	Negative	34 (68%)
	Positive	16(32%)
Hormonal receptor status	Negative	12 (24.0%)
	Positive	38 (76.0%)
HER2	Not assessed	2 (4.0%)
	Negative	28 (56.0%)
	Positive	20 (40.0%)
Distant metastasis	No.	47 (94.0%)
	Yes	3 (6.0%)

Table (3): Comparison between the two studied groups as regard Irisin level

Item	Cases N=50	Controls N=50	P
	Mean ± SD Median (IQR)	Mean ± SD Median (IQR)	
Irisin µg/ml	2.55 ± 0.59 2.37 (1.8- 3.8)	3.57 ± 1.6 3.44 (2.4-0.1)	P<0.001**

P-value was calculated by Mann-Whitney U test

Table (4): Genotype of irisin in cases and controls.

		Cases N=50		Controls N=50		Total		P
		No.	%	No.	%	No.	%	
genotyping	AA	21	42.0%	26	52.0%	47	47.0%	0.6
	AG	24	48.0%	20	40.0%	44	44.0%	
	GG	5	10.0%	4	8.0%	9	9.0%	
alleles	A	66	66%	72	72%	138	69%	0.3
	G	34	34%	28	28%	62	31%	

Table (5): Relationship between genotyping and different prognostic parameters of the cancer cases

		Genotyping						
		AA		AG		GG		
		No.	%	No.	%	No.	%	
Pathological type	invasive ductal	21	44.7%	24	54.5%	2	22.2%	P<0.005**
	invasive lobular	0	0.0%	0	0.0%	3	33.3%	
Grade	*I*	0	0.0%	0	0.0%	3	60.0%	P<0.005**
	II	16	76.2%	21	87.5%	2	40.0%	
	III	5	23.8%	3	12.5%	0	0.0%	
Lymph node status	involved	13	61.9%	19	79.2%	2	40.0%	P=0.17
	not involved	8	38.1%	5	20.8%	3	60.0%	
Distant metastasis	No	21	100%	21	87.5%	5	100%	P=0.17
	yes	0	0.0%	3	12.5%	0	0.0%	

Discussion:

Breast cancer represents the most prevalent malignancy among women across the globe. Although its incidence remains high, advancements in early detection strategies and therapeutic approaches have significantly contributed to lowering mortality rates [9, 10]. Recently, increasing attention has been directed toward irisin, a myokine released from muscle tissue following the cleavage of the FNDC5 gene for its possible role in the pathogenesis and treatment of several diseases, including cancer [3]. At Sohag University Hospital, a study was carried out to explore the association between irisin gene polymorphisms and breast cancer susceptibility, the pattern of allele distribution among patients, and how serum irisin levels correlate with the disease. The study demonstrated that breast cancer patients had considerably reduced serum irisin levels compared to healthy controls. These findings are consistent with a meta-analysis conducted by Flora M. and colleagues in 2022, which reported

diminished irisin gene expression in individuals with cancer. Other investigations [Aydin, S., 2006; Gaggini, M. et al., 2017; Kuloðlu, T. et al., 2019; Kuloðlu, T. et al., 2016] likewise highlighted a reduction in irisin expression within malignant tissues relative to normal ones [11, 12, 13]. Furthermore, Provatopoulou et al. (2015) observed markedly lower serum irisin concentrations in patients with invasive ductal carcinoma of the breast, suggesting its potential value as a reliable disease marker. Their analysis estimated that a one-unit increase in irisin levels may reduce the risk of developing breast cancer by nearly 90% [14]. This is the first study in Sohag to examine the association between single nucleotide polymorphisms of the irisin gene and breast cancer. A significant correlation was identified between genetic profiling and tumor subtype ($p < 0.005$), with the GG genotype being linked to invasive lobular carcinoma, whereas the AA and AG genotypes were associated with invasive ductal carcinoma. Moreover, a strong relationship was also observed between genetic variants and tumor grade ($p < 0.005$), where the GG genotype was mainly related to grade I tumors, while AA and AG genotypes were more frequently connected to grades II and III.

Conclusion:

The study concluded that circulating irisin may play a protective role against breast cancer, as its serum levels were significantly reduced in patients compared with the control group. The findings suggested that although irisin gene polymorphisms may not represent a direct risk factor for developing the disease, they could influence tumor behavior and aggressiveness. Our study has some limitations as small numbers of participants and lack of facilities.

Author's contributions:

First third fourth Author study design, writing
Second Author data collection, writing, revision

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