Lack of association between factor-associated suicide gene-670 A/G polymorphism and risk of breast cancer in Egyptian women

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Received: 10 December 2022
Revised: 11 January 2023
Accepted: 22 January 2023
Published: 28 April 2023

The Egyptian Journal of Surgery 2023, 41:1769–1776

Background
Breast cancer (BC) is among the commonest malignancy and is the leading cause of women's death globally. Factor-associated suicide (FAS) is a key player in the initiation of apoptosis. A to G single nucleotide polymorphism (SNP) at 670 bp in the FAS gene promoter diminishes the interaction of transcription factors with the promoter and the level of FAS expression.
Patients and methods
This study investigated whether the FAS gene promoter A/G SNP at 670 bp increases the risk of BC in Egyptian females. A total of 300 patients with BC and 300 healthy controls were enrolled in this case-control research. To assess genotyping, DNA taken from participants' blood was subjected to PCR-restriction fragment length polymorphism procedure.
Results
FAS-670 A/G genotypes and alleles had no significant association with BC risk (*P* value for genotypes and alleles was 0.250 and 0.164, respectively), but women with the GG genotype who use contraception have a 4.74 times higher risk of developing BC than persons with the AA genotype who do not use contraception. Furthermore, contraceptive users with the GG genotype have a 3.5 times higher chance of developing BC than noncontraceptive users with the same genotype. In addition, the distribution of genotypes and alleles in different BC stages was significant statistically (*P*=0.001).
Conclusion
There is a lack of association between the FAS-670 A/G SNP and BC risk in Egyptian women. According to this study, women with the GG genotype who used contraception had a statistically significant chance of developing BC. Furthermore, the FAS-670 A/G SNP was associated with BC progression in a statistically significant manner.

Keywords:
breast cancer, factor-associated suicide gene, single nucleotide polymorphism

Egyptian J Surgery 41:1769–1776

1110-1121

Introduction

With 0.5 million fatalities and more than two million new cases in 2020 [1], female breast cancer (BC) is one of the most commonly diagnosed cancers and a major cause of mortality among women [2]. The number of new BC cases is expected to increase to more than three million by 2040, according to the International Agency for Research on Cancer (GLOBOCAN) estimates [3]. According to recent reports, developing countries will suffer from two-thirds of new BC cases by 2035 [4]. According to the Egyptian National Cancer Institute (NCI), breast malignancy is the most common cancer affecting Egyptian women, with 28 000 cases diagnosed each year [5]. BC was the most prevalent tumor affecting the participants who attended the Oncology Center of Mansoura University (OCMU) in Egypt in 2016, followed by benign thyroid tumors and leukemia, with the greatest prevalence in the Mansoura City of Dakahlia [6]. The development of BC is influenced by several risk factors. In almost all cases of BC, a combination of hereditary and environmental variables has a significant effect on the development of the disease [7].

The role of apoptosis, or programmed cell death, is critical in a variety of physiological functions, including cell number control and the elimination of undesirable cells during organism development [8]. The intrinsic mitochondrial and the extrinsic pathways are both involved in the apoptotic process [9]. Apoptosis resistance is a characteristic of a variety of cancers induced by structural changes in various apoptotic genes [10].

 DOI: 10.4103/ejs.ejs_297_22

Factor-associated suicide (FAS), type II transmembranous protein, is a member of the tumor necrosis factor family. It functions as a death receptor as it triggers the process of apoptosis by interacting with a specific ligand called the factor-associated suicide ligand (FASL) [11]. FAS has various biological roles that have been addressed in previous studies, such as inflammation, migration, invasion, and proliferation, in addition to its role in apoptosis [12].

The FAS gene, which has nine exons, is localized on human chromosome 10q24.1 [13]. There are multiple polymorphisms in the promoter of the FAS gene, including a 1377 bp G/A alteration and a 670 bp A/G substitution [14]. The binding sites of the signal transducer and activator of transcription 1 and specificity protein 1 transcription factors are disrupted by these polymorphisms, resulting in a decrease in FAS expression, which is critical for the regulation of apoptosis [15].

FASL, type II transmembranous protein, has a homotrimeric structure and belongs to the tumor necrosis family 19 [16]. FASL gene encodes FASL protein. It is found on chromosome 1q23 and has four exons [17].

Numerous studies demonstrated a correlation of FAS-670 A/G single nucleotide polymorphism (SNP) with the prognosis and outcome of lung, breast, and bladder cancers [18,19], as well as with increased risk of colorectal carcinoma [20], BC [21], pharyngeal carcinoma [22], polycystic ovarian syndrome [23], and several autoimmune diseases [24].

This study aimed to clarify whether the FAS-670 A/G SNP is associated with the risk of BC in Egyptian women.

Patients and methods

Research participants

This research was carried out at the Faculty of Medicine Medical Biochemistry and Molecular Biology Department. Participants with BC were recruited from Mansoura Oncology Center University Hospitals in the period between January 2019 and January 2020. A case–control study with 600 participants was conducted. There were 300 individuals who attended the OCMU and were diagnosed with BC; 42 of them had BC stage I, 132 of them had BC stage II, and 126 of them had BC stage III. The healthy women (control group) visited the oncology center as a part of a preventive health checkup (had a risk of breast cancer due to positive family history and they were pathologically free from breast cancer and many came for routine screening and also they were completely free from breast cancer).. Each study participant signed a written consent form.

BC was detected histopathologically in the patient group. TNM and the eighth edition of the American Joint Committee on Cancer staging were used to perform BC staging [25]. Previous malignancy history, as well as previous cancer treatment, such as radiation therapy, hormone therapy, and chemotherapy, were all considered exclusion criteria.

Data about BC cases were gathered from OCMU. Following the routine investigations performed at this center, medical reports were obtained and included a complete history that covered age, family history, marital state, and menstrual history; clinical examination; tissue histopathological investigations; radiological workup; tumor marker assessment; and contraception or hormonal replacement therapy use. The control group consisted of age-matched healthy women without prior history of malignancy and were recruited from the OCMU during a physical assessment.

Ethical approval was obtained from the Institutional Review Board (IRB) of the Faculty of Medicine Mansoura University, Egypt (code number: MS/18.12.419), in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from all participants who participated in the study.

Sample collection

The venipuncture method was used to collect 5 ml of blood samples from the study participants, which were then placed in EDTA tubes, labeled properly, and refrigerated at −80°C for further molecular studies.

DNA extraction

DNA was extracted from whote blood cells using a DNA extraction kit. The concentration of genomic DNA was determined using a NanoDrop 2000 spectrophotometer.

Factor-associated suicide gene polymorphism (rs1800682) genotyping

The FAS gene (rs1800682) SNP was detected using the PCR-restriction fragment length polymorphism technique. Tables 1 and 2 show the amplification PCR primers, restriction enzymes, and digestion

Table 1 Patient and control groups' demographics, as well as clinical and pathological aspects of the cancer patient group

Characteristics	Case number=300	Control number=300	*P* value
Median age	53	53	0.862
BC family history			
Positive	108 (36)	24 (8)	0.001
Negative	192 (64)	276 (92)	
Hormonal contraception			
Positive	102 (34)	108 (36)	0.608
Negative	198 (66)	192 (64)	
Menopausal status			
Premenopausal	108 (36)	216 (72)	< 0.001
Postmenopausal	192 (64)	84 (28)	
BC stage			
Stage I	42 (14)		
Stage II	132 (44)		
Stage III	126 (42)		
Estrogen receptor			
Negative	60 (20)		
Positive	240 (80)		
Progesterone receptor			
Negative	90 (30)		
Positive	210 (70)		
Ki 67 percentage	18 (15–40)		
HER2 score			
0–1+ (negative)	60 (20)		
2+ (borderline)	78 (26)		
3+ (positive)	162 (54)		

Except for age and Ki67 percentage, which are represented in the form of median (interquartile range), data are represented as frequency or percentage. BC, breast cancer. *P* value by χ^2 test.

Table 2 Factor-associated suicide polymorphism primer sequence, restriction enzyme, and digestion pattern

Primers	Primer sequences	Base pair	Restriction enzyme	Genotype
Forward	5'ATAGCTGGGGCTATGCGATT-3'	193 bp	Bme1390I (ScrFI)	AA: 193 bp GG: 136+57 bp AG: 193+136+57 bp
Reverse	5'CATTTGACTGGGCTGTCCAT-3'			

Table 3 Polymerase chain cycling condition

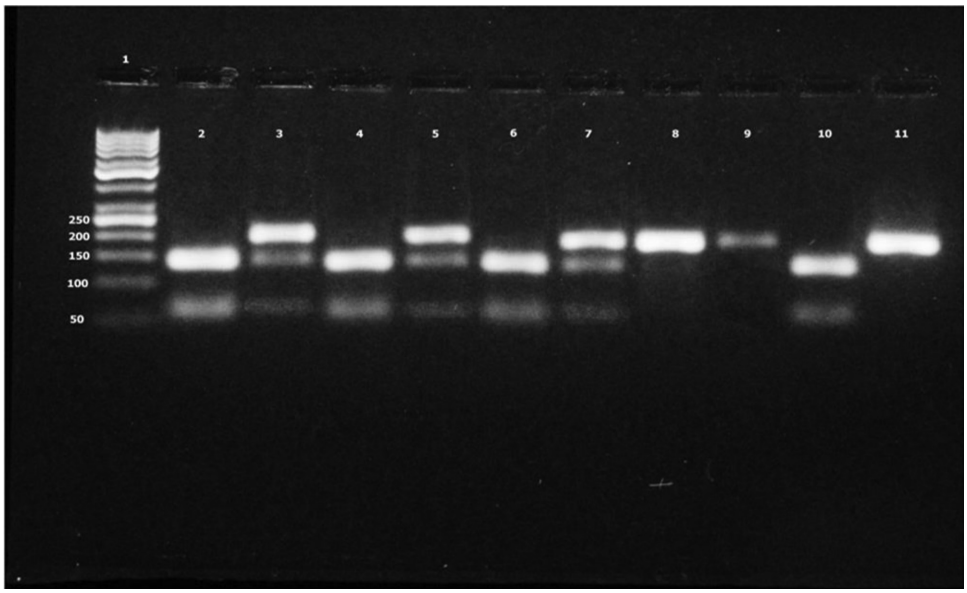
Cycle numbers	Temperature (°C)	Duration
1 initial denaturation cycle	95	2 min
35 cycles of		
Denaturation	95	15 s
Annealing	57	20 s
Extension	72	10 s
1 cycle of the final extension	72	5 min

patterns. The thermal cycler (Applied Biosystems, model 2720, 850 Lincoln Centre Drive, Foster City, California 94404, USA) was tuned for a specific amplification program as described in Table 3. The UV transilluminator was used to visualize the PCR products, and the gel documentation system was used to photograph them. Following digestion, separation of products on a 3% agarose gel was performed using a molecular marker (50–1350 pb) to determine the size of DNA, as illustrated in Figs 1–3.

Statistical analysis

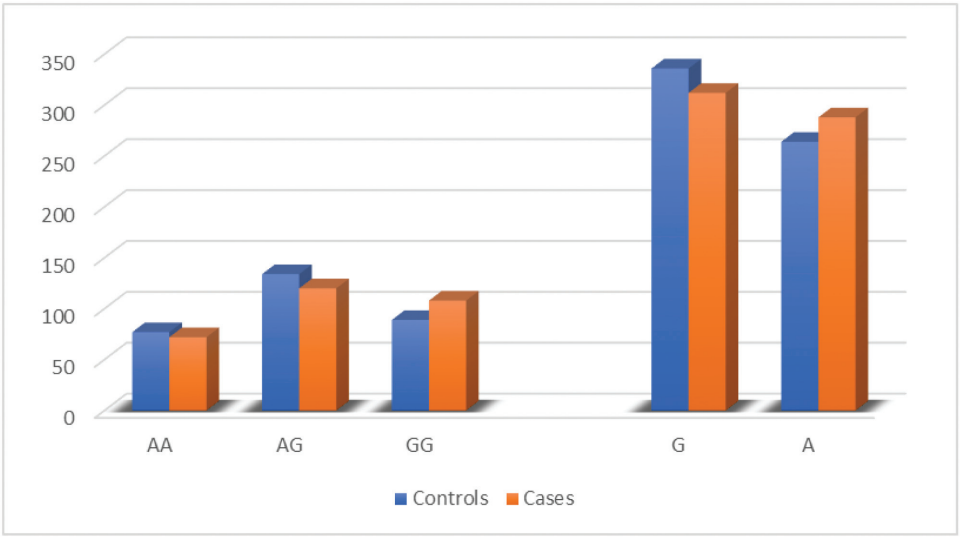
In the statistical analysis was done using IBM SPSS statistics for windows, Version 23.0. Armonk, NY: IBM Corp was employed. The χ^2 test was used to analyze the genotype and allelic frequency of the FAS polymorphism in patients with cancer and control groups. It was also used to determine the association between FAS gene SNP and BC stage. When the *P* values in any of the tests employed were less than 0.05, the results were considered to be statistically significant. The odds ratio (95% confidence intervals) was used to determine the association of FAS genotypes and contraceptive use with BC risk in patients and healthy controls. To assess the consistency of genotype distribution with Hardy–Weinberg equilibrium (HWE), a genotyping exact test of the FAS-670 SNP was performed. The best inheritance model for BC prediction was determined by odds ratio and 95% confidence interval.

Figure 1



FAS-670 A/G SNP analysis through 3% agarose gel electrophoresis following Bme1390I restriction endonuclease digestion. AA genotype is represented by a single band at 193 bp at lanes 8, 9, and 11. GG genotype is represented by two bands at 136, 57 bp at lanes 2, 4, 6, and 10. AG genotype is represented by three bands at 193, 136, and 57 bp at lanes 3, 5, and 7. Lane one represents a 50-base pair molecular ladder. FAS, factor-associated suicide; SNP, single nucleotide polymorphism.

Figure 2



FAS polymorphism genotypes and alleles distribution in patients with cancer and control groups. FAS, factor-associated suicide.

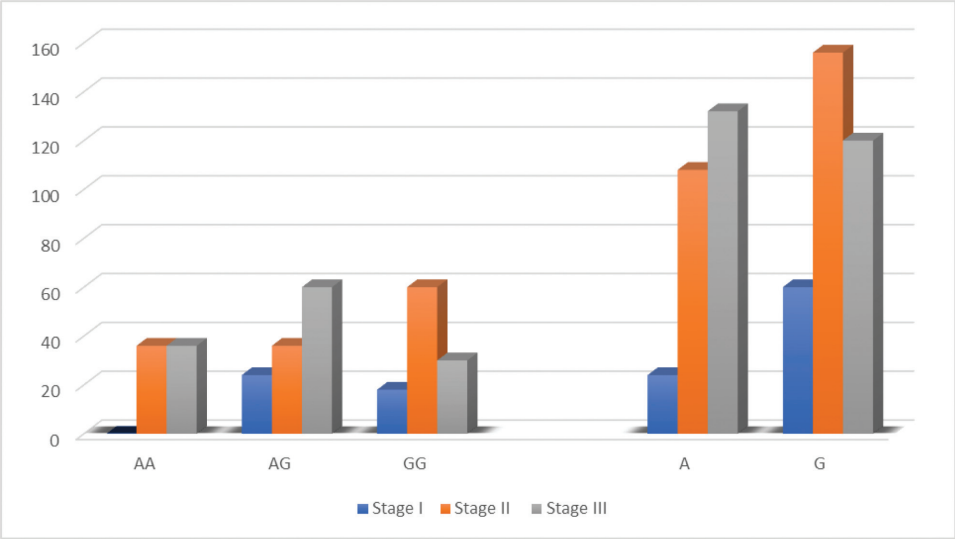
Results

Table 4 shows the genotypes and allele frequencies of FAS SNP in BC cases and controls, demonstrating that there is no statistically significant difference between both groups.

Table 5 displays the association between FAS gene SNP genotypes and hormonal contraceptive use with BC risk. It shows that participants with the GG genotype who use hormonal contraception have a 4.74 times higher odds ratio to develop BC than those with the AA genotype who are non-contraceptive users. Furthermore, contraceptive users with the GG genotype have 3.5 times higher odds ratio to develop BC than noncontraceptive users with the same genotype.

Table 6 also demonstrates a statistically significant difference in the genotypes and alleles distribution in

Figure 3



Association of FAS genotypes and alleles with breast cancer stages. FAS, factor-associated suicide.

Table 4 Factor-associated suicide polymorphism genotypes and allele frequency distribution in patients with cancer and the control group

Genotypes and alleles	Controls	BC cases	χ^2	*P* value
Genotype	*N*=300	*N*=300		
AA	77 (25.7)	72 (24)		
AG	134 (44.7)	120 (40)	2.772	0.250
GG	89 (29.7)	108 (36)		
Allele	*N*=600	*N*=600		
A	288 (48)	264 (44)	1.932	0.164
G	312 (52)	336 (56)		

Data are represented as frequency (percentage). BC, breast cancer. *P* value by χ^2 test.

different cancer stages, indicating a correlation between FAS gene polymorphism and BC progression in the patient group. In concerns of genotypes, the AA genotype was not found in any of the patients of stage I, but it was found in equal numbers in stages II and III. Stage I had a lower prevalence of AG genotype than stages II and III. The GG genotype was found more frequently in stage II than in stages I and III. Regarding alleles, the A allele was found more frequently in BC stage III than in cancer stages I and II, whereas the G allele was found more frequently in BC stage II than in cancer stages I and III.

Table 5 Association of factor-associated suicide genotypes and contraceptive use with breast cancer risk in patient and control groups (*N*=600)

	Negative history of contraceptive usage			Positive history of contraceptive usage		
	Controls	BC cases	Odds ratio (95% CI)	Controls	BC cases	Odds ratio (95% CI)
AA	52	54	1.00	25	18	1.97
A/G	75	84	0.95	59	36	1.00
GG	65	60	1.36	24	48	4.74

Data are represented as frequency. BC, breast cancer; CI confidence interval.

Table 6 Association of factor-associated suicide gene polymorphism with breast cancer stage in the patient group

	Stage I (*N*=42)	Stage II (*N*=132)	Stage III (*N*=126)	*P* value
Genotype				
AA	0 a	36 b (27.3)	36 b (28.6)	<0.001
AG	24 a (57.1)	36 b (27.3)	60 a (47.6)	
GG	18 a, b (42.9)	60 b (45.5)	30 a (23.8)	
Allele				
A	24 a (28.6)	108 a (40.9)	132 b (52.4)	<0.001
G	60 a (71.4)	156 a (59.1)	120 b (47.6)	

Data are represented as frequency and percentage. If the *P* value is less than 0.05, it is considered significant. The *P* value was calculated using the χ^2 test.

Furthermore, HWE was tested in BC cases and control groups, with *P* values of 0.0011 and 0.068 in cancer cases and control groups, respectively, indicating that the reported allele and genotype frequencies of FAS-670 SNP in the control group are compatible with HWE, as described in Table 7.

Additionally, the recessive model, which has the lowest *P* value, Akaike information criterion value, and Bayesian information criterion value, is the statistically significant model used to predict BC (*P*=0.0016, akaike information criterion=601.7, and Bayesian information criterion=628.1), as shown in Table 8. The odds ratio and 95% confidence interval were used to determine it.

Discussion

BC is the most frequent malignancy in women and is the causative factor of the greatest number of cancer-related deaths in women worldwide. BC kills 627 000 people and causes 2.09 million cases globally [26]. BC is a multifactorial tumor, meaning that it develops as a result of a combination of risk factors, including genetics, environmental factors, and lifestyle factors [27].

Table 7 Genotype exact test for Hardy–Weinberg equilibrium of factor-associated suicide A/G single nucleotide polymorphism

Genotype	Total participants (*N*=600)	Case (*N*=300)	Control (*N*=300)
AA	156 (26)	72 (24)	77 (25.7)
AG	240 (40)	120 (40)	134 (44.7)
GG	204 (34)	108 (36)	89 (29.7)
HWE: *P* value	< 0.001	0.0011	0.068

Data are represented in the form of frequency (percentage). HWE, Hardy–Weinberg equilibrium. This table revealed a Hardy–Weinberg equilibrium for the healthy controls (*P*>0.05).

Apoptosis is a type of physiologic cellular death that is critical for maintaining tissue homeostasis. When this mechanism is disrupted, cancer develops [28]. The malignant cell is defined by its resistance to apoptotic stimuli. Distinct human malignant tumors are caused by different apoptosis pathway disruptions [29].

FAS is a death receptor that is involved in apoptosis signaling. It triggers apoptosis by interacting with the FASL/CD95L [20].

FAS and FASL molecules are important in immunological surveillance. Cancer cells express FAS at a lower level, allowing them to escape antitumor cells, whereas these cells express FASL at a higher level, allowing them to counterattack FAS-sensitive tumor-infiltrating lymphocytes [21].

The FAS rs1800682 A/G SNP was found to have no significant association with the risk of BC in the study sample. In comparison with the healthy controls, there was no statistically significant difference in frequencies of genotypes and alleles in patients with cancer. As a result, the FAS-670 SNP is not thought to be a BC predictor. The A allele is present in 48% of the control group and present in 44% of the BC group, whereas 52% of the controls and 56% of patients with BC have the G allele (*P*=0.164). The AA genotype is present in 25.7% of the control group versus 24% of patients with cancer, the AG genotype is present in 44.7% of the healthy group versus 40% of the BC patients, and the GG genotype is present in 29.7% of the control group versus 36% of patient group (*P*=0.250).

In contrast, a study carried out in Zahedan found the FAS-670 SNP was a BC risk factor (odds ratio = 3.181; 95% confidence interval = 1.21–8.33;

Table 8 Analysis of factor-associated suicide single nucleotide polymorphism association with the breast cancer risk among cancer cases and control groups

Model	Genotype	Group		Odds ratio (95% CI)	*P* value	AIC	BIC
		BC case (*N*=300)	Control (*N*=300)				
Codominant	A/A	72	77	R			
	A/G	120	134	0.78 (0.46–1.30)	0.0043	602.8	633.5
	G/G	108	89	1.71 (0.98–2.97)			
Dominant	A/A	72	77	R	0.76	611.6	637.9
	A/G-G/G	228	223	1.08 (0.67–1.72)			
Recessive	A/A-A/G	192	211	R	0.0016	601.7	628.1
	G/G	108	89	2.01 (1.29–3.14)			
Over-dominant	A/A-G/G	180	166	R	0.007	604.4	630.8
	A/G	120	134	0.57 (0.38–0.86)			
Log-additive	–	–	–	1.35 (1.03–1.77)	0.031	607	633.4

Data are presented as count. AIC, akaike information criterion; BC, breast cancer; BIC, Bayesian information criterion; CI, confidence interval; OR, odds ratio; R, reference. *P* value by standard logistic regression.

$P = 0.019$) [21]. Moreover, it was demonstrated in a previous study carried out in China that the FAS rs1800682 SNP has been associated with a higher risk of BC [30]. The study's findings could be attributed to differences in ethnicity, as well as environmental and genetic background.

Our findings are in accordance with those of a prior study, which found no correlation between FAS-670 SNP and BC [31].

In the case group, there was a statistically significantly higher number of women who were postmenopausal and had a family history of BC compared with the control group, with a family history of BC being positive in 38% of patients with BC compared with just 8% of controls. This was in contrast to a previous study that investigated subgroups based on menopausal state [30]. A combination of the GG genotype and the use of hormonal contraception was found to have a statistically significant risk of BC in this study.

Furthermore, there was a statistically significant link between the FAS-670 SNP and various stages of BC. The A allele is present in 28.6% of patients with cancer with stage I, 40.9% of patients with stage II, and 52.4% of patients with stage III, and the G allele is present in 71.4% of patients with cancer with stage I, 59.1% of patients with stage II, and 47.6% of patients with stage III ($P<0.001$).

The AA genotype is present in 0% of patients with cancer with stage I, 27.3% of patients with stage II, and 28.6% of patients with stage III. The AG genotype is present in 57.1% of patients with cancer with stage I, 27.3% of patients with stage II, and 47.6% of patients with stage III. The GG genotype is present in 42.9% of patients with cancer with stage I, 45.5% of patients with stage II, and 23.8% of patients with stage III ($P<0.001$).

A prior study implemented in Tunisia revealed that the FAS-670A/G SNP was a marker of BC progression (odds ratio=2.49, $P=0.03$) [32].

In previous studies, it was shown that FAS-670 polymorphism was correlated with an increased risk of colorectal carcinoma [20], gastric cancer [33], pharyngeal cancer [22], and cervical cancer [34].

The sample size was small, and statistical power was limited to clarify the association. As a result, more research with a bigger sample size is needed to investigate the link between apoptotic receptor genes polymorphisms and BC risk. Furthermore, investigating the gene expression profiles of the FAS gene in blood and tissue samples could aid in the development of a diagnostic and predictive marker for BC.

Conclusion

Our findings revealed no statistically significant association between FAS-670 SNP and risk of BC. On the contrary, individuals with the GG genotype who use hormonal contraception have a statistically significant risk of BC. There was also a statistically significant association between the FAS-670 A/G SNP and the progression of BC.

Acknowledgements

Authors' contributions: all authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by A.A. Z., M.E.N., M.A.A.E., and K.E.S. The first draft of the manuscript was written by A.A.Z., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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