Saadawy *et al.*
Egyptian Journal of Medical Human Genetics (2024) 25:4
https://doi.org/10.1186/s43042-023-00470-6

Egyptian Journal of Medical Human Genetics

RESEARCH

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Diagnostic and prognostic value of single nucleotide polymorphisms in autophagy-related genes (ATG) among Egyptian patients with breast cancer disease

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Abstract

Background Autophagy-related genes (ATGs), associated with autophagy, contribute to the pathogenesis of many illnesses, including cancer. ATGs' role in breast cancer (BC) is still under investigation. Therefore, the current study aimed to determine whether genetic variants in core ATGs correlate with BC prognosis and investigate their impact on protein plasma levels.

Methods This case–control study was carried out on 70 BC patients as well as 70 cancer-free controls in order to determine the association of these variants with BC risk. ATG10 (rs1864182) and ATG7 (rs1375206) polymorphisms were genotyped in whole blood samples using TaqMan SNP Genotyping Assays, and ATG7 and ATG10 levels in plasma were determined using ELISA.

Results The results revealed that ATG7 (rs1375206) might contribute to BC, as patients with the GG genotype displayed a substantial association with BC (OR = 3.23, 95% CI 1.12–9.5) as well as a significant increase in ATG7 protein expression. For ATG7 rs1375206, genotypes GG was significantly associated with increased BC risk; carriers of the G allele frequently have a bad prognosis compared to carriers of the CC genotype (OR of mortality equals 3.01). Serum ATG 7 in the breast cancer patients' group was significantly higher than that in the control group ($p < 0.001$). In contrast, carriers of the ATG10 (rs1864182) CC genotype were significant with a lower risk of BC (OR = 0.31, 95% CI 0.26–0.79) when compared with patients with AA genotype, while serum ATG 10 protein levels were decreased in patients carrying C allele ($p < 0.05$). Carriers of the C allele frequently have a good prognosis (OR of mortality equals 0.79) also the C allele were significantly less likely to have higher grade tumor (14.3% compared to 65.2% of A allele).

Conclusions Single gene polymorphisms (SNPs) within the ATG7 (rs1375206) and ATG 10 (rs1864182) are substantially correlated with BC among Egyptian females. Consequently, SNPs should be considered critical prognostic markers for distinguishing individuals with ATG7 (rs1375206) at elevated risk of developing BC as well as its progression from those with ATG 10 (rs1864182) at lower risk and the effect of these SNPs on its protein expression levels as ATG7 (rs1375206) polymorphism associated with decreased plasma ATG7 level, on the other hand, ATG 10 (rs1864182) polymorphism accompanied with increased ATG 10 plasma level.

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Keywords Breast cancer, ATG7, ATG10, Diagnosis, Prognosis, Single gene polymorphisms (SNPs)

Introduction

BC has resulted in elevated mortality rates, with more than 500,000 women diagnosed with BC worldwide, making it the most common malignancy among women [1]. Female breast cancer has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases. It is the fifth leading cause of cancer mortality worldwide, with 685,000 deaths. Among women, breast cancer accounts for 1 in 4 cancer cases and for 1 in 6 cancer deaths, ranking first for incidence in the vast majority of countries (159 of 185 countries) [2].

The specificity and sensitivity of the current statistical approaches for determining BC risk are limited [3]. SNP genotyping is under investigation to determine its accuracy in stratifying BC risk as well as guiding treatment. Researchers have investigated genetic variation associated with BC risk. In addition to clinic pathologic status, recent findings suggest a correlation between SNPs in specific genes and BC risk [4].

Along with the well-known BRCA2 and BRCA1 mutations that considerably elevate BC risk, several additional moderate as well as low-risk susceptibility variants were found [5], containing the apoptosis-related enzyme caspase-8 (CASP8) [6].

Autophagy, a conserved cellular mechanism, is responsible for replacing defective organelles and improperly folded proteins. Numerous biological processes, including the onset of senescence and the maintenance of genomic stability, are regulated by autophagy, which is essential for cellular homeostasis [7].

The core group of autophagy-associated genes (ATG) in mammals is largely preserved, reflecting their significant intracellular function across numerous animals. Autophagy genes are frequently linked to the pathophysiology of several diseases, such as cancer, autoimmune illnesses, and neurodegenerative disorders, due to their crucial role in the maintenance of mammalian cells [7].

Cellular stresses can activate autophagy through a variety of regulatory pathways. The core ATG proteins facilitate the formation and induction of autophagosomes, allowing for their fusion with lysosomes and subsequent degradation [8, 9].

In one of the crucial stages of autophagy induction, the phagophore, a double-membrane structure, forms. The phagophore and developing autophagosome are augmented with lipidated LC3/GABARAPs that attract interacting particles. P62/SQSTM1, also known as Sequestosome-1, is a protein that functions as an autophagy receptor. By physically interacting with the autophagosome, this protein expedites the destruction of ubiquitinated cargo during autophagy. Consequently, genetic variations in the ATG genes influence autophagy and cause specific disorders [7].

Lapidation of LC3 is essential for the formation of autophagosomes, and the ATG7 protein plays a crucial role in this process. It adenylates ATG12, leading to the formation of ATG5-ATG12 conjugates. Additionally, ATG7 adenylates LC3-I to facilitate its lipidation into LC3-II [8, 9].

Autophagy is implicated in various diseases, including cancer, and is believed to have both tumor-suppressing and tumor-promoting effects in cancer [10]. The role of autophagy in cancer depends on the context and stage of carcinogenesis, which may help to explain its paradoxical impacts [11].

although much research has been done on autophagy proteins and breast cancer but, the exact association between ATG 7 and ATG 10 gene polymorphisms with breast cancer in the Egyptian population; and whether they influence their serum Protein levels and clinical outcome are not yet fully understood. So in our research we highlighted how ATG7 (rs1375206) and ATG 10(rs1864182) are substantially correlated with BC among Egyptian females.

Materials and methods

Subjects

The current case–control study included a control group ($n=70$) of healthy individuals from the same population with no family history of cancer. Additionally, this study included 70 females who had BC. The Zagazig University Institutional Review Board approved this study, and all procedures complied with the Helsinki Declaration. All participants completed consent forms before interviews and sample collection (patients and controls). Then trained interviewers gathered personal data on demographics, menstrual and reproductive history. Then, a sample of 10 ml of venous blood in an EDTA tube was taken. (Divided into 2 tubes, one 5 ml for ELISA, the other 5 ml for genotype analysis), in the General Surgery Department at Zagazig University Hospital, Egypt.

ELISA for plasma ATG7 and ATG10

Within 30 min after sample collection, centrifugation was performed for 15 min at 1000 ×*g* at 2–8 °C. For testing, the supernatant was gathered and stored at −80 °C. For the analysis of the ATG7 and ATG10 proteins, the

frozen plasma samples were allowed to thaw completely at an ambient temperature before being thoroughly mixed by a vortex and centrifuged to extract the supernatants. Human Autophagy Related Protein 7 (ATG7) (My BioSource, CA, USA Catalog #MBS062423) and Human Ubiquitin-Like-Conjugating Enzyme ATG10 (ATG10) ELISA Kits (My BioSource, CA, USA Catalog #MBS9318935) were used to test the levels of ATG7 and ATG10 in the plasma of 70 patients and 70 controls, respectively. The assay was completed as per the manufacturer's guidelines. Based on the kit standard, plasma levels of ATG7 and ATG10 were estimated, and the data were reported as the mean ± SD.

DNA extraction and genotyping

The QIAamp DNA Mini Kit (QIAamp Blood Kit-Qiagen GmbH-Hilden-Germany: Cat. No./ID: 51104) was utilized for DNA extraction to perform genotyping from whole blood samples of both BC patients and controls by the manufacturer's instructions. For use in subsequent investigations, DNA samples with absorbance values (A260/280 nm) of 1.5 to 2.0 were kept in a refrigerator at − 80 °C. ATG7 (rs1375206) and ATG 10 (rs1864182) polymorphism were achieved using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA; SNP Assay ID C_11953870_70 for ATG 10 (rs1864182) and SNP Assay ID C_1288835_10 for ATG7 (rs1375206)) and Reactions were performed in a StratageneMX3005P Real-Time PCR System with the following protocol: the thermal cycling conditions were set as initial denaturation for 1 cycle at 95 °C for 5 min, followed by 45 cycles of denaturation and final extension of 95 °C for 10 s and 60 °C for 30 s, respectively.

Data analysis

The current work underwent statistical analysis using the 22nd version of the SPSS software (SPSS Inc-USA-Chicago). Data were expressed as mean ± SD for quantitative variables, frequency and percentage for categorical variables. Chi-squared (X^2) and t test were used when appropriate. Odd`s ratio was calculated to assess the risk. p value < 0.05 was considered statistically significant.

Results

(1). Demographic characteristics of cases and controls:

Subjects' clinical as well as demographic characteristics are depicted in Table 1**.** One-hundred and forty subjects, including 70 BC patients, averaging 50.3 ± 9.4 years (mean age at menarche 13.3 ± 1.5 years), and 70 healthy controls, averaging 51.4 ± 8.9 years (mean age at menarche 13.3 ± 1.5 years) were recruited for this study.

Table 1 Demographic and clinical characteristics of cases and control

	Cases		Controls		*p* value
	N **= 70**		***N*** **= 70**		
Age (years)					
X ± SD	50.3 ± 9.4		51.4 ± 8.9		0.68
Age at menarche (years)					
X ± SD	13.3 ± 1.5		13 ± 1.7		0.84
Menopausal status	*N*	%	*N*	%	
Premenopausal	38	54.3	37	52.9	
Post-Menopausal	32	45.7	33	47.1	0.3
Eestrogen receptor (ER)	*N*	%			
Negative	24	34.3			
Positive	46	65.7			
Progesterone receptor (PR)	*N*	%			
Negative	16	22.9			
Positive	54	77.1			
Nodule metastasis	*N*	%			
No	26	37.1			
Yes	44	62.9			
HER2 status	*N*	%			
Negative	48	68.6			
Positive	22	31.4			
Clinical stage	*N*	%			
I/II	30	42.9			
III/IV	40	57.1			
Chemotherapy or radiotherapy	*N*	%			
No	18	25.7			
Yes	52	74.3			

X ± SD (Mean ± standard deviation)

In the patient group, there were 54.3% premenopausal and 45.7% menopausal women; in the healthy control group, there were 52.9% premenopausal and 47.1% menopausal women. There was no significant difference in age distribution and menopausal state between controls and patients ($p > 0.05$). Among the cases, 65.7% had ER positivity, 77.1% had PR positivity, and 31.4% had HER2 positivity. Additionally, 62.9% of cases had nodal metastasis, and 74.3% received radiotherapy or chemotherapy.

(2). Associations between ATG7 rs1375206 and ATG10 rs1864182 SNPs in selected ATG genes with breast cancer risk

Table 2 depicts the genotype distributions of the 2 SNPs as well as their correlation with BC risk. In controls, the reported genotype frequencies for these SNPs were consistent with the Hardy–Weinberg equilibrium. The ATG7 SNP (rs1375206) G allele was considerably correlated

Table 2 Associations between 2 SNPs in selected ATG genes with breast cancer risk

	SNP	Location	Allele[a]	Case[b], *N* = 70	Control [b], *N* = 70	MAF[c] (case/control)	HWE[d]	OR (95%CI)	*p*
ATG7	(rs1375206)	Intron	C/G	26/23/21	32/30/8	0.464/0.329	0.9	1.77 (1.06–2.96)	0.02*
ATG 10	(rs1864182)	Missense	A/C	46/17/7	31/24/15	0.22/0.386	0.154	0.75 (0.59–0.93)	0.01*

[a] Major/minor allele

[b] Major homozygote/heterozygote/rare homozygote between cases and controls

[c] Minor allele frequency

[d] Hardy–Weinberg equilibrium test among controls

*significant *p* value < 0.05

with a higher risk of BC than controls (OR = 1.77, $p = 0.02$).

Conversely, the C allele of rs1864182 (ATG10) was substantially linked to deceased BC risk (per-allele OR = 0.75, $p = 0.01$).

According to the findings in Table 3, Carriers with rs1375206 GG genotype were more widespread in BC cases (30%) compared to controls (11.4%). There was a significant association between the rs1375206 GG genotype and elevated BC risks in subjects (OR = 3.23, 95% CI 1.12–9.5). In contrast, in ATG10 rs1864182, the frequency of the CC genotype was significantly higher among healthy controls compared to cancer patients (21.4% Vs. 10%). Carriers of the CC genotype were at significantly lower risk of developing BC than the AA genotype (OR = 0.31, 95% CI 0.26–0.79)

(3). Genotype and Allele frequencies of ATG7 rs1375206 and ATG10 rs1864182 SNPs and their correlation with clinical status in BC patients.

As regards the relation between ATG7 (rs1375206) and demographic and clinical factors, the only significant associations were found with ER as patients with one G allele in the rs1375206 SNP (OR 2.2; 95% CI 1.04–5.04), were significantly more likely to have positive estrogen receptors than C allele, and also a significant association with treatment as patients with one G allele in the rs1375206 SNP (OR 5.2; 95% CI 1.96–14.4), were significantly more likely to undergo chemotherapy or radiotherapy (Table 4). Furthermore, ATG10 (rs1864182) genotype is shown in Table 5 clarified that there were statistically significant associations between Menarche age (years) and the Clinical stage of the tumor with ATG10 (rs18 64182) distribution of the alleles. (p-value < 0.05). Concerning menarche, Carriers of the C allele were significantly less likely to have their menarche at >14 years more than those with the A allele (OR = 0.23, $p < 0.05$). Also, carriers of the C allele were significantly less likely to have T3 and T4 tumor stages than those with the A

Table 3 Association of ATG7 (rs1375206) and ATG 10 (rs1864182) genotype and allele frequency with breast cancer

	Cases *N* = 70		Controls *N* = 70		OR (95% CI)	*p* value
	N	%	*N*	%		
ATG7(rs1375206)						
CC	26	37.1	32	45.7	1 (reference)	*p* value < 0.05
CG	23	32.9	30	42.9	0.94 (0.42–2.14)	
GG	21	30.0	8	11.4	3.23 (1.12–9.5)*	
Allele						
C	75	53.6	94	67.1	1 (reference)	
G	65	46.4	46	32.9	1.77 (1.06–2.96)*	
ATG10(rs1864182)						
AA	46	65.7	31	44.3	1 (reference)	*p* value < 0.05
CA	17	24.3	24	34.3	0.48 (0.21–1.1)	
CC	7	10	15	21.4	0.31 (0.1–0.95)*	
Allele						
A	109	77.9	86	61.4	1 (reference)	
C	31	22.1	54	38.6	0.45 (0.26–0.79)*	

*significant *p* value < 0.05

Table 4 Association between ATG7 (rs1375206) demographic and clinical characteristics of breast cancer

	CC *N* = 26		CG *N* = 23		GG *N* = 21		C/G	OR (95%CI)
	N	%	*N*	%	*N*	%		
Age (years)								
≤ 50	16	61.5	14	60.6	10	47.6	46/ 29	1
> 50	10	38.5	9	39.1	11	52.4	34/31	1.45 (0.7–3.00)
Menarche age (years)								
≤ 14	19	73.1	12	52.2	15	71.4	50/25	1
> 14	7	26.9	11	47.8	6	28.6	42/23	1.11 (0.51–2.43)
Menopause								
No	16	61.5	12	47.8	11	52.4	43/32	1
Yes	10	38.5	13	52.2	10	47.6	33/32	1.3 (0.63–2.59)
Clinical stage								
I/II	13	50	9	39.1	8	38.1	35/40	1
III/IV	13	50	14	60.9	13	61.9	25/40	1.4 (0.67–2.91)
Node metastasis								
No	13	50	7	30.4	6	28.6	33/42	1
Yes	13	50	16	69.6	15	71.4	19/46	1.9 (0.89–4.09)
ER status								
Negative	14	53.8	4	17.4	6	28.6	32/43	1
Positive	12	46.2	19	82.6	15	71.4	16/49	2.2 (1.04–5.04)*
PR status								
Negative	9	42.3	2	8.7	5	23.8	20/55	1
Positive	17	65.4	21	91.3	16	76.7	12/53	1.61 (0.67–3.09)
HER2 status								
Negative	11	42.3	5	21.7	6	28.6	27/48	1
Positive	15	57.7	18	78.3	15	71.4	17/48	1.59 (0.72–3.51)
Chemotherapy or radiotherapy								
No	13	50 50	3	13	2	9.5	29/46	1
Yes	13		20	87	19	90.5	7/58	5.2 (1.96–14.4)*

*significant *p* value < 0.05

allele (OR = 0.32, $p < 0.05$) highlighting their possible protective role.

(4). Association of genotype variants with BC patient survival

Our study revealed that ATG7. G allele was upregulated in death (poor survival) (OR = 3.01). Meanwhile, the ATG10 C allele was down-regulated in deaths (good survival) (OR = 0.79), but the association was not statistically significant (Table 6).

(5). Association of ATG7 rs1375206 and ATG10 rs1864182 SNPs and their plasma level in BC

Genetic variants frequently influence an individual's susceptibility to disease by altering protein expression levels. ATG7 and ATG10 protein expression level in both groups is shown in Fig. 1. To evaluate the effect of ATG7 and ATG10 on the generation and development of BC, 70 patients and 70 controls were chosen from the SNP-tested sample for the plasma ATG7 and ATG10 protein assay by ELISA. In ATG7, mean plasma levels were considerably elevated in BC patients than in controls. In contrast, ATG10 exhibited markedly diminished levels in cases than controls ($p < 0.001$).

The correlation study of genotype-protein expression indicated that plasma ATG7 levels significantly differed between people with the GG genotype and individuals with the GC and CC genotype of rs1375206 ($p < 0.001$). Individuals with the CC genotype displayed the highest level of 11.1 ± 0.43. Conversely, the plasma level of ATG10 considerably decreased when comparing individuals with the AA genotype to those with the CA and CC genotypes, with the lowest level in individuals with the CC genotype (2.67 ± 0.15) o ($p < 0.05$) (Fig. 2).

Table 5 Association between ATG10 (rs1864182) genotype and demographic and clinical

	AA *N* = 46		CA *N* = 17		CC *N* = 7		A/C	OR (95% CI)
	N	%	*N*	%	*N*	%		
Age (years)								
≤ 50	25	54.3	9	52.9	6	85.7	59/21	1
> 50	21	45.7	8	47.1	1	14.3	50/10	1.78 (0.71–4.5)
Menarche age (years)								
≤ 14	36	78.3	8	47.1	2	28.6	80/12	1
> 14	10	21.7	9	52.9	5	71.4	29/19	0.23 (0.09–0.59)*
Menopause								
No	27	58.7	5	29.4	6	85.7	59/17	1
Yes	19	41.3	12	70.6	1	14.3	50/14	1.03 (0.43–2.47)
Clinical stage								
I/II	16	34.8	8	47.1	6	85.7	40/20	1
III/IV	30	65.2	9	52.9	1	14.3	69/11	0.32 (0.13–0.79)*
Node metastasis								
No	17	37	5	29.4	4	57.1	39/13	1
Yes	29	63	12	70.6	3	42.9	70/18	1.3 (0.53–3.15)
ER status								
Negative	17	37	5	29.4	2	28.6	89/9	1
Positive	29	63	12	70.6	5	71.4	70/22	0.73 (0.28–1.89)
PR status								
Negative	11	23.9	3	17.6	2	28.6	25/7	1
Positive	35	76.1	14	82.4	5	71.4	84/24	0.98 (0.34–2.76)
HER2 status								
Negative	16	34.8	3	17.6	3	42.9	35/9	1
Positive	30	65.2	14	82.4	4	57.1	74/22	0.86 (0.33–2.23)
Chemotherapy or radiotherapy								
No	14	30.4	3	17.6	1	14.3	31/5	1
Yes	32	69.6	14	82.4	6	85.7	78/ 26	0.48 (0.15–1.49)

*significant *p* value < 0.05

Table 6 Association between genotype and survival of breast cancer patient

	Death		Survival		OR (95% CI)
	N	%	*N*	%	
ATG 7					
CC	2	20	24	47.5	1 (reference)
CG	3	30	20	35.6	1.8 (0.21–5.4)
GG	5	50	16	16.9	3.75 (0.13–8.41)
C/G	7/13		68/42		1/3.01 (0.5–4.62)
ATG 10					
AA	8	72.7	39	66.1	1 (reference)
CA	2	18.2	14	23.7	0.7 (0.09–4.29)
CC	1	9.1	6	10.2	0.84 (0.12–5.73)
A/C	18/4		92/26		1/0.79 (0.2–2.77)

Discussion

BC is responsible for 14% of all cancer-related deaths in women worldwide and 23% of all new cancer cases in women [12]. BC prognosis is significantly influenced by the disease stage during diagnosis. It can be induced by multiple environmental and genetic factors as BC has complex and varied etiology [13]. Genetic testing and screening rates should be increased for hereditary BC for early diagnosis.

SNPs are a helpful tool for examining the etiology, variations in treatment responses across individuals, and outcomes of malignancies. SNPs were identified as potential disease risk factors [14]. Research on several human malignancies has focused on genetic differences in the autophagy core genes [15]. Several polymorphic variations have been discovered and associated with the start of a number of diseases, including coronary

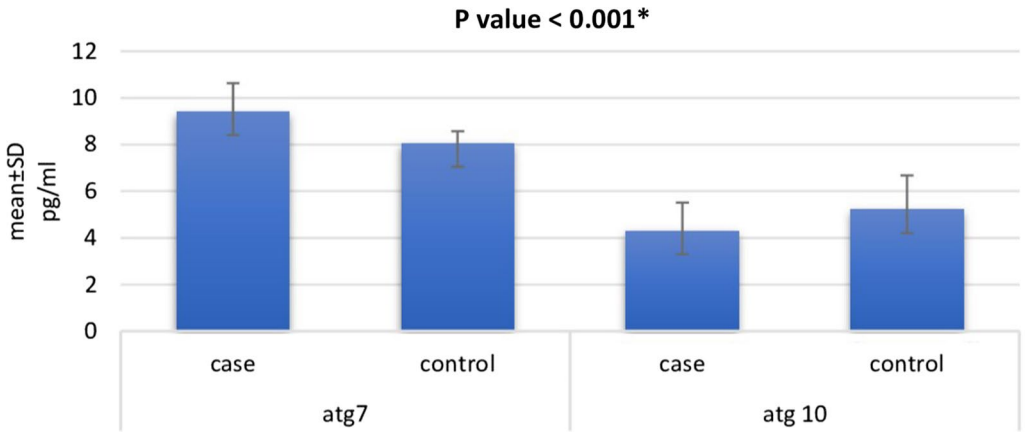

Fig. 1 Plasma levels of ATG7 and ATG10 by ELISA test

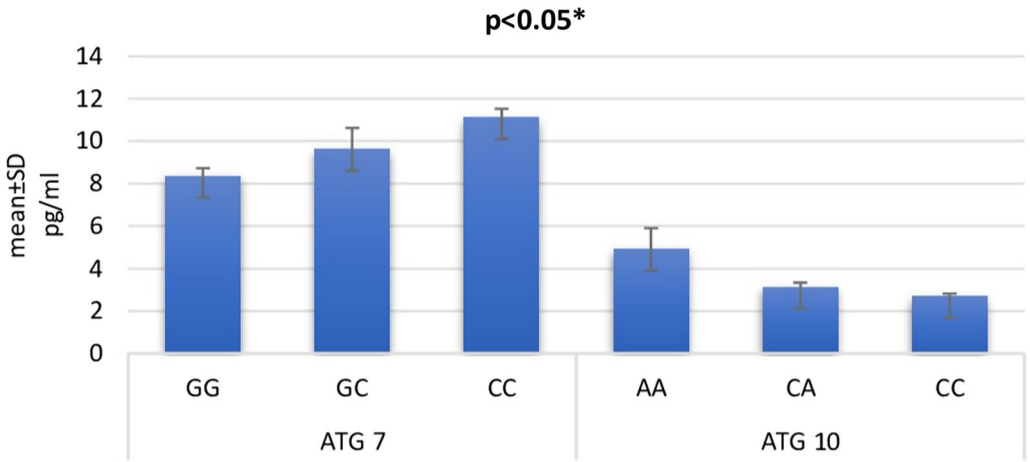

Fig. 2 Plasma level according to genotype in cases

artery disease [16], Parkinson's disease [17] and Huntington's disease [18].

This case–control study, which includes 70 cases and 70 controls, was carried out to assess the relationship between autophagosome formation polymorphisms and BC susceptibility as well as the prognosis of BC patients. These mutant alleles contribute to higher cancer risk by exhibiting more aggressive characteristics, thereby increasing the size of the tumor and stage and spreading to surrounding lymph nodes and other regions. Two SNPs, one in ATG 7 (rs1375206) and the other in ATG 10 (rs1864182), contributed to increasing or decreasing cancer risk in Egyptian female patients.

The frequency of ATG 7 (rs1375206) genotype polymorphism differed significantly between cases and controls in analyses that controlled for potential confounding factors, and the G allele of ATG7 SNP (rs1375206) was significantly associated with a higher risk of BC when compared with the control. (OR = 1.77). However, there was a considerably higher likelihood of receiving radiotherapy or chemotherapy if there was one G allele in the rs1375206 SNP. The fact that the G allele was upregulated in death (OR = 3.01) in a study examining the relationship between the ATG7 genotype and survival of BC patients may be due to the limited sample size. Our findings suggest that the ATG 7 rs1375206 SNP may be a BC susceptibility gene. However, little research has been done on its potential contribution to BC carcinogenesis.

Sarosh et al. conducted a study to investigate the role of ATG rs 1375206 in patients with coronary artery disease and found a significant association of *ATG7* rs1375206 and rs550744886 polymorphism with CAD [20]. Zhao et al. also studied the role of Associations of ATG7 rs1375206 polymorphism and elevated plasma ATG7 levels with late-onset sporadic Parkinson's disease [21]. Their study suggested that the rs1375206 polymorphism in *ATG7* may not be associated with late-onset sporadic PD; however, high plasma

ATG7 levels and the A-T haplotype may be associated with susceptibility to late-onset sporadic PD in the Han population from Zhejiang and Guangdong provinces which agree with our result.

In harmony with our results, Zhou et al. reported that ATG7 overexpression had a poor average survival of BC patients, consistent with our findings. These results demonstrated the significance of ATG7 in BC patients' survival and may aid in the early detection of patients with a bad prognosis [19].

Evidence suggests that ATG7 is involved in the development of tumors and might be used as a potential target in cancer treatment. ATG7 is upregulated in several cancer tissues, including breast, prostate, and colorectal [20–22] The BC metastasis process may also be significantly impacted by the active expression of ATG7 [23]. ATG7 knockdown significantly slowed the in vivo tumor development in triple-negative BC by preventing autophagy [24]. ATG7 deficiency was also shown to have anti-cancer properties in genetically modified mice designed to mimic pancreatic and [14] lung cancer [25, 26]. These pieces of evidence confirm our recent discovery that ATG7 protein overexpression may worsen the prognosis for BC patients by encouraging cancer spread or relapse.

The ATG 10 (rs1864182) variant is anticipated to be located at exonic splicing enhancers (ESEs), according to the web resource SNPinfo [27]. By altering the nucleotides in the target ESEs, SR proteins may no longer bind with them, preventing the spliceosome machinery from skipping exons [28]. As a result, missense or nonsense mutations in the protein-coding sequences of splicing enhancer sequences may also change those sequences and result in abnormal splicing. Evidence suggested a connection between exon-skipping events in BC and the BRCA1 mutation [29–31]. Therefore, we hypothesized that this missense variant might result in a catalytic modification of ATG10, which would then affect autophagosome formation and alter BC risk.

According to the current investigation results, the polymorphism of ATG 10 (rs1864182) is linked to a lower risk of BC. Our result demonstrated a substantial inverse relationship between BC risk and the C allele of rs1864182 (ATG10) concerning the demographic and clinical characteristics of this SNP with significantly associated with more favorable conditions as carriers of the C allele were significantly less likely to have their menarche at > 14 years and to have T3, and T4 tumor stages more than those with the A allele. Survival analysis of the ATG10 proved that the C allele was down-regulated in deaths. Our findings suggested ATG10 might be a protector against BC, but further research may be needed on a larger population.

ATG10 rs1864182 has previously been linked to a lower risk of adult acute myeloid leukaemia (AML) [32] and melanoma [33], consistent with our findings. Contrary to our findings, Qin et al., in their research on the Chinese population, demonstrated that ATG10 might be a BC susceptibility gene [34]. In contrast to our results, earlier research about ATG10 rs1864182 has been linked to poor survival in lung cancer cases, particularly in non-small cell lung cancer (NSCLC) [35]. Also, ATG10rs1864182 was also identified as a biomarker for acquired or primary chemotherapy resistance while utilizing the EGFR-TKI drug (gefitinib) in advanced lung cancer cases with EGFR (epidermal growth factor receptor) mutations [34]. Exploring ATG10 rs1864182's role in chemotherapy resistance may lead to the development of a helpful biomarker for BC treatment in the future.

After highlighting ATG7 SNP (rs1375206) polymorphism connection to BC, the impact of ATG 7 SNP associated with BC on the protein expression level of ATG7 isoform was assessed in the serum of cases and controls, revealing that ATG7 mean plasma levels in BC patients were considerably elevated than in controls. The CC genotype showed the greatest level.

These results indicated that the ATG7 mutations could change ATG7's protein level and inhibit autophagy. This finding is in line with what has been observed in Parkinson's disease, where functional studies have proven that sequence variants within the ATG7 gene promoter may alter the expression of the ATG7 protein, thereby impacting autophagy activity, and act as a risk factor for the development of Parkinson's disease [36]. Consistent with our findings, another study on Chinese newborns with cerebral palsy discovered that the levels of plasma ATG7 were elevated in CP patients, further demonstrating the link between CP and ATG7 [37].

We investigated the potential effects of the sequence variants on the level of protein expression in both healthy people and BC patients. We were able to identify changes in the ATG10 protein level, with lower levels in the presence of the ATG10 rs1864182 mutation and the lowest level in people with the CC genotype. Li et al. findings illustrated that the lower ATG10 protein levels in ATG10rs1864182 carriers were linked to decreased autophagic flux and canonical function, aligning with our data [38].

The current investigation provided evidence that the genetic polymorphism mutations of ATG 7 at rs1375206 are linked to elevated BC risk among Egyptian females, with controversy ATG 10 at rs1864182 associated with decreased risk of BC. Additionally, this study shows links between pathological and clinical aspects and the ATG 7 and ATG 10 gene polymorphisms and the effect of these polymorphisms on their protein level expression as

ATG7, mean plasma levels were considerably elevated in BC patients than in controls. In contrast, ATG10 exhibited markedly diminished levels in cases than controls. These polymorphisms can be used to provide molecular detection by acting as prognostic and predictive indicators for BC onset.

Conclusions

To sum up, the ATG7 SNP (rs1375206) G allele was considerably correlated with a higher risk of BC than controls, associated with a bad prognosis and increased mortality. In ATG7, mean plasma levels were considerably elevated in BC patients than in controls. We also concluded that ATG10 rs1864182 is associated with a decreased incidence of BC, and carriers of the C allele were significantly less likely to have higher tumor stages than those with the A allele. In contrast, ATG10 exhibited markedly diminished plasma levels in cases compared to controls, highlighting their possible protective role. Our findings offer novel explanations for how genetic differences in ATG10 and ATG7 may coordinate the development of BC. The gathered data could be used to create preventative plans or evaluate individuals with other BC risk factors.

Abbreviations

AML	Acute myeloid leukaemia
ATGs	Autophagy-related genes
BC	Breast cancer
ELISA	Enzyme-linked immunosorbent assay
SNPs	Single gene polymorphisms
BRCA2 and BRCA1	Breast cancer gene 1, 2
CASP8	Caspase-8
LC3	Light chain 3 protein
GABARAPs	γ-Aminobutyric acid receptor-associated proteins
P62/SQSTM1	Sequestosome-1
EDTA	Ethylenediamine tetraacetic acid
LSD	Least significant difference
SD	Standard deviation
ANOVA	One-way analysis of variance
ESEs	Exonic splicing enhancers
NSCLC	Non-small cell lung cancer
EGFR	Epidermal growth factor receptor

Acknowledgements

We acknowledged all subjects included in our study.

Author contributions

WO and SFS prepared the idea and designed the study. SFS did the data statistical analysis. WO and SFS performed all the laboratory investigations and interpreted the patients' data regarding each studied group. AR selected the patients and the control group. All authors wrote, read, and approved the final manuscript.

Funding

No funding was received.

Data availability

Data are available from the corresponding author upon reasonable request due to privacy/ethical restrictions.

Declarations

Ethics approval and consent to participate

This study has been approved by the Faculty of Medicine, Zagazig University, IRB, for human studies (reference number is IRB#:10222-7-12-2022), and the patients have signed informed written consent.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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Received: 7 August 2023 Accepted: 23 December 2023
Published online: 05 January 2024

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