Egyptian Journal of Medical Microbiology Volume 34 / No.3 / July 2025 217-227 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Association between the RETN Polymorphisms and Breast Cancer Susceptibility

[1]Hanan J. Nayyef*, [2]Ismail H. Aziz
[1]Genetic Engineering and Biotechnology Institute, Baghdad University, Iraq; Tropical Biological Research Unit/College of Science, Baghdad University, Iraq
[2]Genetic Engineering and Biotechnology Institute, Baghdad University, Iraq

Key words:
RETN, BC, Resistin

Corresponding Author:
Hanan Jawad Nayyef
Tropical Biological Research Unit/College of Science, Baghdad University/ Iraq;
Department Genetic Engineering and Biotechnology Institute, Baghdad University, Iraq
Tel.: 009647901884906
hanan.nayyef@uobaghdad.edu.iq
ORCID ID:
https://orcid.org/0000-0002-4340-1184

ABSTRACT

Background: *Resistin (RETN), a cysteine-rich adipokine secreted by macrophages or adipose tissue, is expressed via the RETN gene with single nucleotide polymorphisms in 3'-untranslated and RETN promoter locations.* ***Objective:*** *To estimate the relationship between Breast Cancer (BC) in Iraqi patients and RETN single nucleotide polymorphisms.* ***Methodology:*** *A study involving 100 women with BC at Al-Amal Hospital in Iraq analyzed RETN nucleotide polymorphisms from June to October 2023.* ***Results:*** *Comparison of RETN (rs10401670 T/C) genotypes and alleles between patients with BC and HCs revealed that TC and CC genotypes had significant relations with BC ($p< 0.001$ and $p < 0.001$, respectively); the rates of TC and CC genotypes were higher in patients in comparison with HCs, 53 versus 34 and 25 versus 10, respectively, therefore both of them acts as a risk factor with odds ratios of 3.97 (2.06 -7.64) and 6.36 (2.63 - 15.40), respectively. The TT genotype was significant protective factor against BC with a ratio of odds: 0.22 (0.12 -0.41) and ($p< 0.001$); allele analysis revealed that allele T was significant protector with a ratio of odds of 0.35 (0.23 -0.53) and ($p<0.001$).* ***Conclusion:*** *RETN (rs10401670 T/C) is the only SNP that has a significant impact on cancer risk. Other SNPs, rs3745369 G/C, rs368504053 T/C, and rs74489689 A/G, were not significantly associated with BC.*

INTRODUCTION

Worldwide, people deal with Breast Cancer (BC). Female BC currently accounts for approximately 25% of all female cancers, and there will be a predictable 2.3 million novel patients in 2020, making it the most common malignancy among women[1]. In 2021, invasive BC will affect an estimated 281,550 women and 2,650 men in the US, whereas ductal carcinoma in situ will affect an additional 49,290 women. From approximately 1 in 11 in 1975 to 1 in 8, the lifetime likelihood of a woman diagnosed with BC has climbed[2].

Created via adipose tissue or continuously released via macrophages, Resistin is a short 108-amino acid peptide that is cysteine-rich and an adipokine (~12.5 KDa hormone). It is encoded via the *RETN* gene. It is created via macrophages, leukocytes, and other tissues and has roles in insulin sensitivity, glucose homeostasis, and adipogenesis. Adipocyte differentiation induces *RETN* expression, which is downregulated in mature adipocytes and is related to *RETN* overexpression. *RETN* has been associated with several malignancies, including colorectal, breast, and prostate cancers. The 3'-untranslated region and the *RETN* promoter have single nucleotide polymorphisms. Cancer of the breast, colon, and metabolic syndrome are among the many illnesses linked to variations in the *RETN* locus[3,4].

Single nucleotide polymorphism (SNP) genotyping has the potential to enhance the risk prediction of BC and disease treatment, according to researches[3,5]. BC susceptibility is affected by certain SNPs[4].

The aim of our research is to estimate the relationship between breast cancer in Iraqi patients and the *RETN* single nucleotide polymorphism. The type of BC used in this study was invasive ductal carcinoma. and Luminal B (Estrogen (ER)/ Progesterone (PR)+, Her2+) was seen in 28 %, luminal A (ER/PR+, Her2-) was seen in 44 %, her2+ (ER/PR−, Her2+) was seen in 8%, and triple negative (ER/PR−, Her2-) was reported in 20 % of cases. The selection of these SNPs likely reflects their potential roles in cancer biology, prior evidence linking them to breast cancer, and relevance to the study population.

METHODOLOGY

This study included 100 females with Breast Cancer (BC) who attended Al-Amal Hospital / Baghdad/Iraq, from June to October 2023. The experimental work was performed at the Institute for Genetic Engineering and Biotechnology Institute for Postgraduate Studies/ University of Baghdad and laboratories of Al-Amal Hospital. The parameters were evaluated using questionnaires and medical records, including location,

age, ER, tumor stage, family history, PR, type, disease grade, and human epidermal growth factor receptor 2 (HER2) receptors.

A BC verified diagnosis based on imaging, physical examination, and histology was an inclusion criterion. The female participants were 20 years of age or older. Individuals who had previously been exposed to hormone treatment, chemotherapy, radiation, or any other kind of cancer, as well as those who suffered from chronic inflammatory disorders, were not eligible to participate and they are in inclusion criteria. One hundred healthy females were age-matched with BC patients to rule out their effect on the genetic results. The study selection criteria included female participants aged 20 years and older, generally in good health without chronic or acute medical conditions, no current medication use, reproductive health, known hormonal disorders, or psychiatric disorders. Lifestyle factors include a balanced lifestyle without substance abuse, irregularities in diet, or exercise patterns. Exclusion criteria included chronic diseases, medications affecting health, pregnancy or breastfeeding, significant mental health disorders, hormonal imbalances or disorders, severe obesity or underweight, lifestyle issues, and infectious diseases or acute illnesses. Each patient and control participant provided descriptive information to complete a questionnaire designed for this purpose.

Sample collection:

Four mL of peripheral blood from the patient and HCs were drawn into a gel tube and the blood was kept to coagulate for approximately 15-20 minutes. After centrifuging the coagulated blood for 15 minutes at 3000 rpm, the serum recovered from each person was split into: The DNA extraction process began with 4 ml of whole blood transferred to an EDTA tube, and the remaining serum was used for the enzyme-linked immunosorbent assay (ELISA) to detect CEA and resistin levels in the sera.

Blood DNA extraction:

Whole Blood DNA MiniPrep Kit, manufactured by ELK Biotechnology CO., Ltd (China EQ002) was used to isolate total genomic DNA from blood with the anti-coagulant EDTA

Genotyping for RETN gene:

Genotyping was performed by extracting DNA from the samples, which were then subjected to polymerase chain reaction (PCR), DNA sequencing of DNA and sequence analysis. Agarose gel electrophoresis was performed to validate amplification after PCR amplification. The extracted DNA criteria were the only determinants of the PCR reliability. The molecular analysis for *RETN* single nucleotide polymorphisms SNPs included rs3745369 G/C, rs368504053 T/C, rs74489689 A/G, and rs10401670 T/C. Macrogen provided the lyophilized primers, which were created using the NCBI-BLAST primer creation program.

Statistical Analysis:

The study utilized SPSS Version 25 statistical software and System-SAS (2012) program for statistical analysis. Pearson's $\chi 2$-test, Hardy-Weinberg equilibrium, logistic regression, SHesis software for haplotype analysis, and Chi-square test for significant comparisons were used to assess relationships between categorical variables.

RESULTS

The sequence of the primers used in this study was presented in table 1

Table 1: Primers used in the present study

Description	Sequence (5'→3' direction)	size (bp)	Optimized temperature	Country/ Company
	rs3745369 G>C (*RETN* gene)		**55**	
Forward	**GTCCACGCTCCTGTGTTC**	**438**		
Reverse	**TTTCGGAGGAAGCAGTTGG**			
	rs10401670 T>C *(RETN* gene)		**55**	
Forward	**GCAA AGGGTGGTCATTCA**	**706**		**Korea / Macrogen**
Reverse	**GGACATCTCAGCATCTGTTC**			

A comparison of the mean age between the patients and controls is presented in Table 2.

Table 2: Comparison of mean age between patients and HCs

Characteristic	**BC group** ***n* = 100**	**HCs** ***n* = 100**	***p-value***
Age (years)			
Mean ±SD	54.86 ±10.77	54.61 ±9.53	0.862 I
Range	27 -75	30 -75	NS

NS, not significant; ***n***, number of cases; **I**, independent sample *t*-test.

Single nucleotide polymorphism (SNP) (rs3745369 G/C) of *RETN* gene

The Hardy–Weinberg equilibrium (HWE) detected non-significant differences in the total cases in the HCs and BC groups (Table 3). Regarding all enrolled cases, the GG genotype was seen in 84 subjects, the GC genotype was observed in 66 subjects, and the CC genotype was seen in 50 subjects. In the BC group, the GG genotype was observed in 28 subjects, the GC genotype in 42 subjects, and the CC genotype in 30 subjects. Regarding HCs, the GG genotype was seen in 24 subjects, the GC genotype was observed in 56 subjects, and the CC genotype was seen in 20 subjects.

Table 3: The Hardy Weinberg equilibrium in total cases and in patients and HCs

***RETN* (rs3745369 G/C)**	**Total *n* = 200**		**BC group *n* = 100**		**HCs *n* = 100**	
	Observed count	**Expected count**	**Observed count**	**Expected count**	**Observed count**	**Expected count**
GG	52	51.0	28	24.0	24	27.0
GC	98	100.0	42	50.0	56	49.9
CC	50	49.0	30	26.0	20	23.0
χ^2	0.0792		2.549		1.483	
p	0.778 NS		0.110 NS		0.223 NS	

RETN: resistin; **NS:** not significant

Figure 1 shows the DNA sequence chromatogram of SNP *rs3745369* in *RETN* gene. A comparison of r *RETN* (rs3745369 G/C) genotypes and allele frequencies among the BC and HCs groups is shown in Table 4. With respect to the co-dominant model, the GG genotype was considered as a reference, and both the GC and CC genotypes showed no significant relationships with BC ($p > 0.05$). No significant relationships between the illness and GG genotype of GG was observed in the dominant model (p = 0.519), and no such relationships were seen in the recessive model (p = 0.102). Allele analysis also revealed no significant relations ($p = 0.548$).

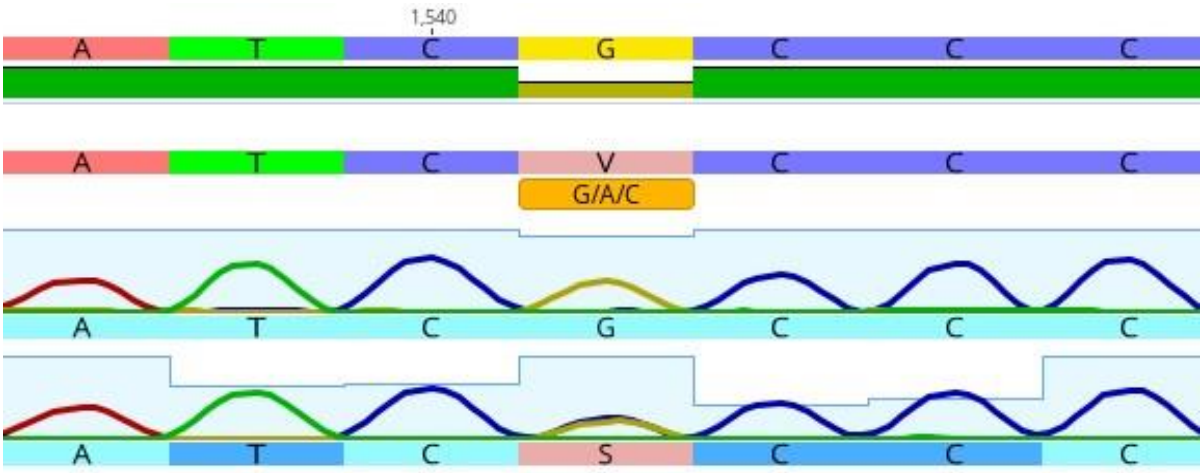

Fig. 1: DNA sequence chromatogram of SNP *rs3745369* of *RETN* gene utilizing Sanger sequencing. One of the alleles is homozygous for G represented as single peak of "G". Having a single "C" peak means that one of alleles is C. A G/C heterozygous allele is shown by the existence of the peaks of "G" and "C".

Table 4: Comparison of *RETN* (rs3745369 G/C) genotypes and alleles frequency between patients with BC and HCs

Model	*RETN* (rs3745369 G/C)	BC group *n* = 100	HCs *n* = 100	*p-value*	OR (95% CI)
Co-dominance	GG	28	24	Reference	Reference
	GC	42	56	6.4e-1	0.64 (0.33 -1.26)
	CC	30	20	3.39e-1	1.29 (0.59 -2.82)
Dominant	GG	28	24	2.3e-1	1.23 (0.65 -2.32)
	GC+CC	72	76	Reference	Reference
Recessive	CC	30	20	1.7e-1	1.71 (0.89 -3.29)
	GG+CC	70	80	Reference	Reference
Allele	G	98	104	8.9e-1	0.89 (0.60 -1.31)
	C	102	96	1.1e-1	1.13 (0.76 -1.67)

OR, odds ratio; **, significant at $p \leq 0.01$; NS, not significant; **C**, chi-square test; **CI**, confidence interval; ***n***, number of cases.

Single nucleotide polymorphism (SNP) (rs10401670 T/C) of *RETN* gene

Figure 2 shows the molecular size of the DNA bands (706 bp) ofof*rs10401670 SNP* for *RETN* gene of patients after amplification by PCR. The HWE detected non-significant differences between the HCs and BC groups, as represented in Table 5. Regarding all enrolled cases, the TT genotype was observed in 78 subjects, the TC genotype was observed in 87 subjects, and the CC genotype was observed in 35 subjects. In the BC group, the TT, TC, and genotype were observed in 22, 53, and 25 subjects, respectively. Regarding HCs, the TT genotype was observed in 56 subjects, the TC genotype in 34 subjects, and the CC genotype in 10 subjects.

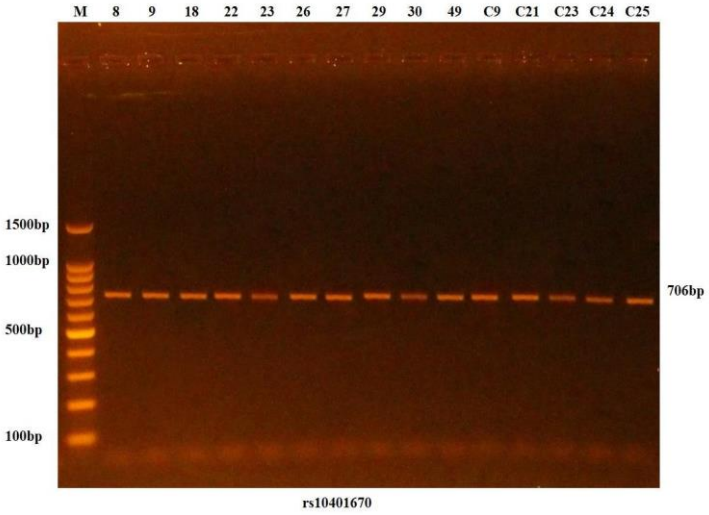

Fig. 2: The molecular size of DNA bands (706 bp) of *rs10401670 SNP* for *RETN* gene of patients after amplification by PCR and these bands were checked under the UV-light after the red safe staining on (1.5%) of gel of agarose at (1.30 hour at 70 volt). Lane M: ladder of DNA (100 bp). Lane (1-15): BC patients.

Table 5: The Hardy Weinberg equilibrium in total cases and in patients and HCs

RETN (rs10401670 T/C)	Total *n* = 200		BC group *n* = 100		HCs *n* = 100	
	Observed count	Expected count	Observed count	Expected count	Observed count	Expected count
TT	78	73.8	22	23.5	56	53.3
TC	87	95.4	53	50.0	34	39.4
CC	35	30.8	25	26.5	10	7.3
χ^2	1.543		0.372		1.890	
p	0.214 NS		0.542 NS		0.169 NS	

***: significant at $p \leq 0.001$; **RETN:** resistin; **NS:** not significant

Figure 3 shows the DNA sequence chromatogram of SNP *rs10401670* in *RETN* gene.

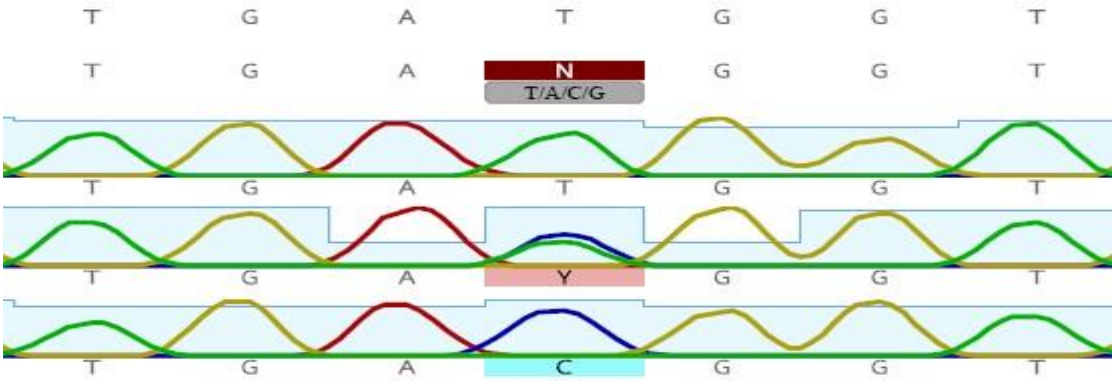

Fig. 3: DNA sequence chromatogram of SNP *rs10401670* of *RETN* gene utilizing sequencing of Sanger. A T/C heterozygous allele is shown by the existence of the peaks of "T" and "C". Having a single "C" peak means that one of your alleles is C. Having a single "T" peak means that one of your alleles is T.

Comparisons of *RETN* (rs10401670 T/C) genotypes and alleles among the BC and HCs groups are presented in Table 6. With respect to the co-dominant model, the TT genotype was considered as a reference, and both TC and CC genotypes detected significant relationships with BC ($p< 0.001$ and $p < 0.001$, respectively), and the ratios of TC and CC genotypes were higher in patients in comparison with HCs, 53 *versus* 34 and 25 *versus* 10, respectively; therefore, both genotypes act as risk factors with odds ratios of 3.97 (2.06 -7.64) and 6.36 (2.63 -15.40), respectively).

Table 6: Comparison of *RETN* (rs10401670 T/C) genotypes and alleles frequency between patients with BC and HCs

Models	*RETN* (rs10401670 T/C)	BC group *n* = 100	HCs *n* = 100	*p*	OR (95% CI)
Co-dominance	TT	22	56	Reference	Reference
	TC	53	34	1e-4	3.97 (2.06 -7.64)
	CC	25	10	1e-4	6.36 (2.63 -15.40)
Dominant	TT	22	56	1e-4	0.22 (0.12 -0.41)
	TC+CC	78	44	Reference	Reference
Recessive	CC	25	10	5e-3	3.00 (1.35 -6.64)
	TT+TC	75	90	Reference	Reference
Allele	T	97	146	1e-4	0.35 (0.23 -0.53)
	C	103	54		2.87 (1.89 -4.36)

NS, not significant; **C**, chi-square test; **CI**, confidence interval; ***n***, number of cases; **, significant at $p \leq 0.01$; **OR**, odds ratio

In the dominant model, TT genotype was significant protective factor against BC ($p< 0.001$) with a ratio of odds (95% CI) of 0.22 (0.12 -0.41); whereas, in the model of recessive, CC genotype was significant risk factor ($p< 0.001$) with an odds ratio of 3.00 (1.35 -6.64). Allele analysis revealed that allele T was a significant protector ($p< 0.001$) with a ratio of odds of 0.35 (0.23 -0.53) and allele C was a significant risk factor ($p< 0.001$) with a ratio of odds of 2.87 (1.89 -4.36).

Single nucleotide polymorphism (SNP) (rs368504053 T/C) of *RETN* gene

The HWE in all cases, patients, and HCs is presented in Table 7. Regarding all enrolled cases, the TT, TC, and genotype were observed in 86, 81, and 33 subjects, respectively. In the BC group, the TT, TC, and genotype were observed in 38, 41, and 21 subjects, respectively. Regarding HCs, the TT, TC, and genotype were observed in 38, 50, and 12 subjects, respectively.

Table 7: The Hardy Weinberg equilibrium in total cases and in patients and HCs

***RETN* (rs368504053 T/C)**	**Total *n* = 200**		**BC group *n* = 100**		**HCs *n* = 100**	
	Expected count	**Observed count**	**Expected count**	**Observed count**	**Expected count**	**Observed count**
TT	80.0	86	40.3	48	39.7	38
TC	93.0	81	46.4	31	46.6	50
CC	27.0	33	13.3	21	13.7	12
χ^2	3.319		10.973		0.526	
p	0.068 NS		<0.001 ***		0.468 NS	

***: significant at $p \leq 0.001$; **RETN:** resistin; **NS:** not significant

Figure 4 shows the DNA sequence chromatogram of SNP *rs368504053* in *RETN* gene.

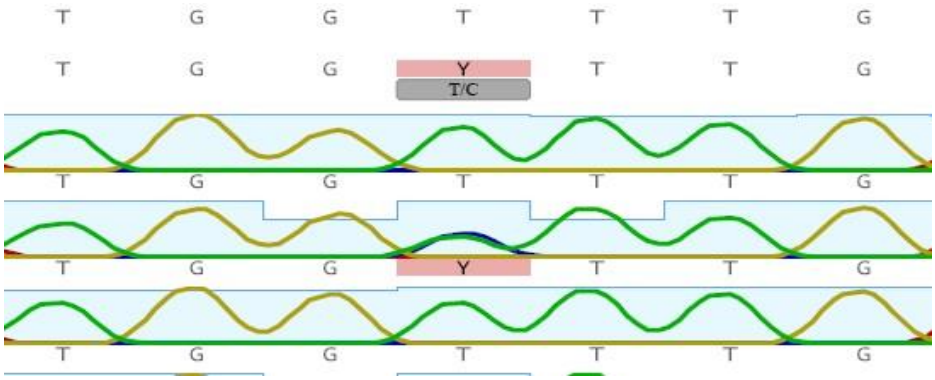

Fig. 4: DNA sequence chromatogram of SNP *rs368504053* of *RETN* gene utilizing Sanger sequencing. T/C heterozygous allele is shown by the existence of the peaks of "T" and "C". Having a single "C" peak means that one of your alleles is C. Having a single "T" peak means that one of your alleles is T.

A comparison of *RETN* (rs368504053 T/C) genotypes and alleles between patients with BC and HCs is presented in Table 8. With respect to the co-dominant model, TT genotype was considered as a reference, and TC genotype detected significant relationships with BC (p = 0.023); the rates of TC genotype was lower in patients in comparison with HCs, 35 *versus* 50);, therefore it acts as a defensive parameter with odds ratios (95%CI) of 0.49 (0.26 -0.91). The CC genotype was not significantly associated with disease (p = 0.439).

In the dominant model, the TT genotype was not significantly related to disease (p = 0.153), and in the recessive model, the CC genotype was not significantly related to disease (p = 0.086). Allele analysis revealed that neither allele T nor allele C had a significant relations with disease (p> 0.05).

Table 8: Comparison of *RETN* (rs368504053 T/C) genotypes and alleles between patients with BC and HCs

Mode	***RETN* (rs368504053 T/C)**	**BC group *n* = 100**	**HCs *n* = 100**	***p-value***	**OR (95% CI)**
Co-dominance	TT	48	38	Reference	Reference
	TC	31	50	2.3e-2	0.49 (0.26 -0.91)
	CC	21	12	4.39e-1	1.39 (0.61-3.17)
Dominant	TT	48	38	1.53e-1	1.51 (0.86 -2.64)
	TC+CC	52	62	Reference	Reference
Recessive	CC	21	12	8.6e-2	1.95 (0.90 -4.22)
	TT+TC	79	88	Reference	Reference
Allele	T	127	126	9.17e-1	1.02 (0.68 -1.53)
	C	73	74	9.8e-1	0.98 (0.65 -1.47)

NS, not significant; **C**, chi-square test; **, significant at $p \leq 0.01$; **OR**, odds ratio; **CI**, confidence interval; ***n***, number of cases.

Single nucleotide polymorphism (SNP) (rs74489689 A/G) of *RETN* gene

The HWE in all cases, patients, and HCs is presented in Table 9. Among all enrolled patients, the GG genotype was observed in 79 subjects, the GA genotype in 77 subjects, and the AA genotype in 44 subjects. In the BC group, the GG, GA, and genotype were observed in 45, 33, and 22 subjects, respectively. Regarding HCs, the GG genotype was observed in 34 subjects, GA genotype in 44 subjects, and AA genotype in 22 subjects.

Table 9: The Hardy Weinberg equilibrium in total cases and in patients and HCs

***RETN* (rs74489689 G/A)**	**Total *n* = 200**		**BC group *n* = 100**		**HCs *n* = 100**	
	Observed count	**Expected count**	**Observed count**	**Expected count**	**Observed count**	**Expected count**
GG	79	69.0	45	37.8	34	31.4
GA	77	96.9	33	47.4	44	49.3
AA	44	34.0	22	14.8	22	19.4
χ^2	8.460		9.189		1.148	
p	0.004 **		0.002 **		0.284 NS	

RETN: resistin; **NS:** not significant;***, significant at $p \leq 0.001$.

Figure 5 shows the DNA sequence chromatogram of SNP *rs74489689* in *RETN* gene.

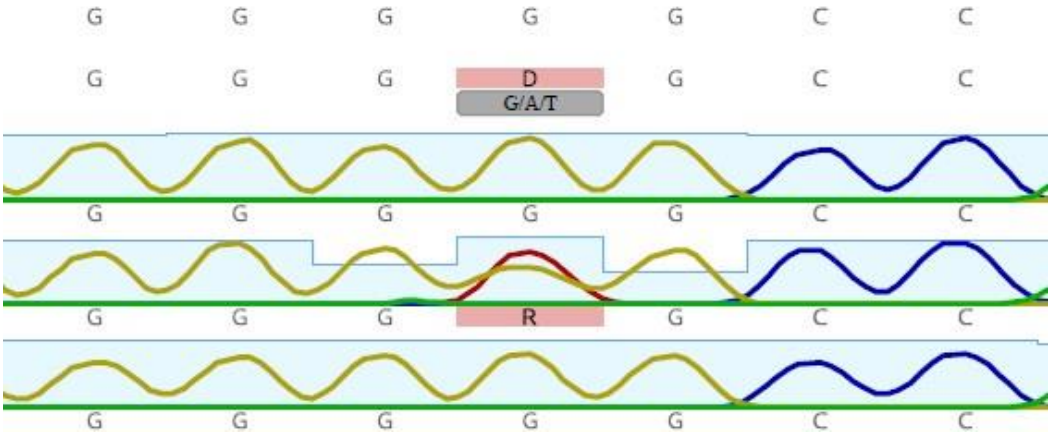

Fig. 5: DNA sequence chromatogram of SNP *rs74489689* of *RETN* gene utilizing Sanger sequencing. The peaks of "G" and "A", when present, indicate that the G/A allele is heterozygous. A single "A" peak indicates that one of the A genes is homozygous. A single "G" peak indicates that one of the alleles is homozygous for G.

A comparison of *RETN* (rs74489689 G/A) genotypes and allele frequencies between patients with BC and HCs is presented in Table 10. With respect to the co-dominant model, the GG genotype was considered as a reference, and both the GA and AA genotypes showed no significant relationships with BC ($p > 0.05$). In the dominant model, the GG genotype was not significantly related to BC (p=0.112), and in the recessive model, the AA genotype was not significantly related to the disease (p = 1.000). Allele analysis revealed that alleles A and G were not significantly associated with the disease (p = 0.264).

Table 10: Comparison of *RETN* (rs74489689 G/A) genotypes and alleles frequency between patients with BC and HCs

Mode	*RETN* (rs74489689 G/A)	BC group *n* = 100	HCs *n* = 100	*p*	OR (95% CI)
Co-dominance	GG	45	34	Reference	Reference
	GA	33	44	5.7e-1	0.57 (0.30 -1.07)
	AA	22	22	7.6e-1	0.76 (0.36 -1.58)
Dominant	GG	45	34	1.59e-1	1.59 (0.90 -2.81)
	GA+AA	55	66	Reference	Reference
Recessive	AA	22	22	1.00e0	1.00 (0.51 -1.95)
	GA+GG	78	78	Reference	Reference
Allele	G	123	112	1.26e-1	1.26 (0.84 -1.87)
	A	77	88	8.0e-1	0.80 (0.53 -1.19)

NS, not significant; **C**, chi-square test; **, significant at $p \leq 0.01$; **OR**, odds ratio; **CI**, confidence interval; ***n***, number of cases.

Haplotype analysis and linkage disequilibrium

The estimated numbers and frequencies of haplotypes (rs3745369, rs10401670, rs368504053, and rs74489689) of the *RETN* in BC patients and HCs are shown in Table 11. For (rs3745369, rs10401670, rs368504053, and rs74489689) SNPs, the C-C-C-A combined effect was significantly related to BC (p = 0.026) with a ratio of odds of 1.622 (1.058-2.486); thus, this combined effect can be considered as a risk factor for the disease. The C-T-C-A and C-T-T-A combined effects were limited to HCs, and none of the patients had such a combination; the differences were significant (p< 0.001), making these effects defensive parameters; however, the odds ratio was not calculated because of mathematical limitations. The G-C-T-G combined effect was limited to the BC group, and none of the controls had such a combination; the difference was significant (p< 0.001), making these effects a risk factor; however, the odds ratio was not calculated because of mathematical limitations.

Table 11: Estimated numbers and frequencies of haplotypes (rs3745369, rs10401670, rs368504053, rs74489689) of the *RETN* gene in BC patients and controls

Haplotypes	Patients *n* = 100		Control *n* = 100		OR	95 % CI	*p-value*
	n	Frequency	*n*	Frequency			
C-C-C-A*	73.00	0.365	54.00	0.270	1.622	1.058-2.486	1.622*
C-T-C-A*	0.00	0.00	20.00	0.100	-	-	5.93e-6
C-T-T-A*	0.00	0.00	11.96	0.060	-	-	**5.00e-4**
C-T-T-G*	5.04	0.025	6.04	0.030	0.852	0.257-2.827	0.852
G-T-T-G*	91.96	0.460	105.96	0.530	0.790	0.531-1.176	0.790
G-C-T-G*	23.00	0.115	0.00	0.00	-	-	**5.73e-7**

All frequencies <0.03 were ignored in the analysis

Linkage disequilibrium analysis between SNPs of *RETN* gene and the corresponding D' and r^2 values are presented in Table 12 and Figure 6.

Table 12: Linkage disequilibrium analysis between SNPs of *RETN* gene

Linkage disequilibrium	rs3745369	rs10401670	rs368504053	rs74489689
rs3745369		**D′0.703** **$r^2$0.407**	**D′ 1.000** **r^2 0.739**	**D′ 0.967** **r^2 0.835**
rs10401670	**D′ 0.703** **r^2 0.407**		**D′ 0.760** **r^2 0.520**	**D′ 0.698** **r^2 0.448**
rs368504053	**D′ 1.000** **r^2 0.739**	**D′ 0.760** **r^2 0.520**		**D′ 1.000** **r^2 0.828**
rs74489689	**D′ 0.967** **r^2 0.835**	**D′ 0.698** **r^2 0.448**	**D′ 1.000** **r^2 0.828**	

r^2: Correlation coefficient of each pair of SNPs (0-1). **D′:** A scaled D value, where D is the interval between -1 and 1, reflecting the linkage disequilibrium for each pair of SNPs.

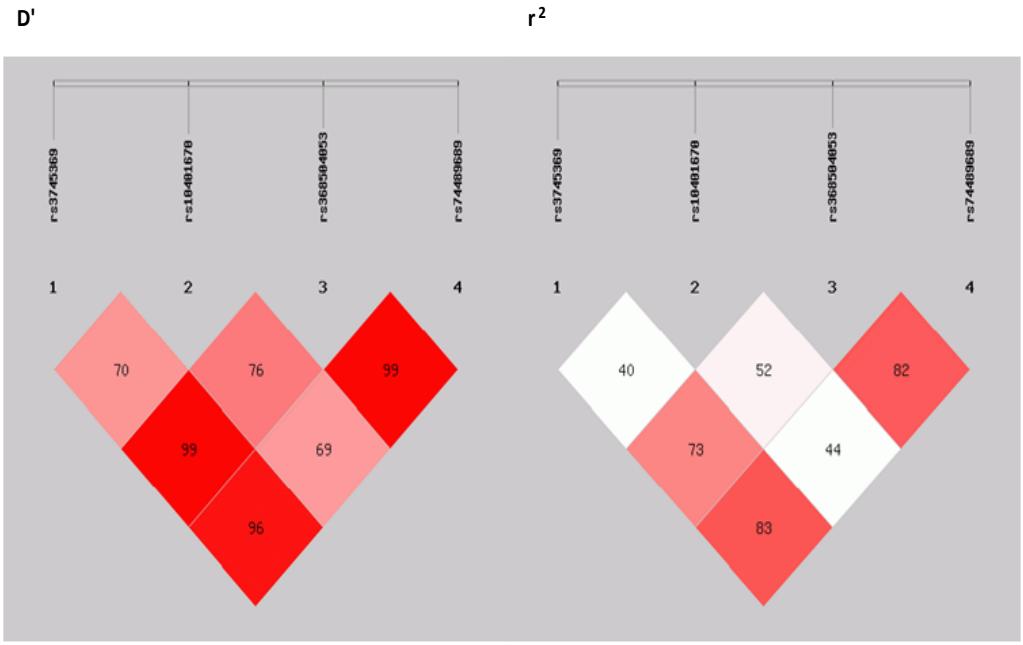

Fig. 6: Pairwise linkage disequilibrium coefficient (D') and correlations coefficient (r^2) between *RETN* SNPs (rs3745369, rs10401670, rs368504053, rs74489689) in BC patients and controls.

DISCUSSION

As part of this research, the Hardy Weinberg equilibrium in patients with Breast cancer (BC) and HCs for rs3745369 G/C detected no significant difference between observed counts and expected counts, indicating at least no significant impact of gene polymorphism on the incidence of BC in Iraqi individuals. This assumption was further supported by the results of genotype analysis, which revealed no significant relationships between any of the genotypes GG, GC, or CC, or even the alleles G and C with BC. In addition, this study reported no significant relationship between genotype and clinicopathological characteristics or serum tumor marker levels. In line with current study observation, Liu *et al.*[6] reported no significant relations between *RETN* (rs3745369 G/C) gene polymorphism and risk of BC "odds ratio (95% CI) was 1.085 (0.945–1.247) and p-value was 0.246".

The Hardy-Weinberg equilibrium analysis for *RETN* (rs10401670 T/C) revealed no significant differences between the expected and observed counts in both patients and healthy controls; however, genotype and allele analysis revealed that genotypes TC, CC, and C were risk factors for BC and that TT genotype and allele T were protective factors against BC. In addition, this study reported no significant relationship between genotypes and clinicopathological characteristics or serum levels of tumor markers. Following an extended search of the available published articles in the internet database, the researcher failed to find an article linking gene polymorphism of *RETN* (rs10401670 T/C) SNP to BC; therefore, to the best of our knowledge, this is the first research attempt to link such SNP gene polymorphism of *RETN* gene with risk and incidence of BC, and this is a point of originality in this thesis.

The SNP (rs368504053 T/C), showed the Hardy Weinberg equilibrium in the group detected significant differences between expected counts and observed counts of patient groups, implying a primary suggestion of the existence of an effect of genotypes or alleles on the risk of BC in Iraqi women. Genotype and allele analyses revealed that the TC genotype was a protective factor, whereas the rest of the genotypes or alleles were not significantly related to the disease. Additionally, this study reported that miR-373 levels were significantly lower in the CC genotype. Following an extended search of the available published articles in the internet database, the researcher failed to find an article linking gene polymorphism of *RETN* (rs368504053 T/C) to BC; therefore, to the best of our knowledge, this is the first research attempt to link such SNP gene polymorphism of *RETN* gene with risk and incidence of BC, and this is a point of originality in this study.

RETN (rs74489689 A/G), the Hardy Weinberg equilibrium in patient groups, detected significant differences between expected counts and observed counts, implying a primary suggestion of the existence of an effect for genotypes or alleles in the risk of BC in Iraqi women; however, genotype and allele analysis failed to prove such an association, and none of the genotypes or alleles detected significant relationships

with the disease; however, this study found that *RETN* levels detected a trend of reduction from GG toward GA and then toward AA genotype. Following an extended search of the available published articles in the internet database, the researcher failed to find an article linking gene polymorphism of *RETN* (rs74489689 A/G) to BC; therefore, to the best of our knowledge, this is the first research attempt to link such SNP gene polymorphism of *RETN* gene with risk and incidence of BC, and this is a point of originality in this study.

In this study for haplotype analysis, the C-C-C-A and G-C-T-G combinations were risk factors for the disease, whereas the C-T-C-A and C-T-T-A were protective factors. The effects of RETN on BC have been investigated in previous studies. RETN is highly expressed in BC tissues and may be a biomarker for the disease stage and degree of inflammation[7].

Research has shown that RETN exerts pro-inflammatory effects by increasing the levels of pro-inflammatory cytokines via the NF-κB signaling pathway, which causes inflammation and carcinogenesis. Low-grade systemic inflammation is one characteristic of obesity.[8,9.10]

Resistin may influence the growth and spread of tumors through various mechanisms. Studies have shown that Resistin activates NF-kB signaling, which regulates TNF-α and IL-12 production. By triggering the PI3K/Akt pathway, which in turn activates matrix metalloproteinase and VEFG through the MAPK (ERK1/2 and p38) pathway, retin promotes neoangiogenesis[11,12] Resistin is implicated in VEFG production, neoangiogenesis, epithelial cell proliferation, matrix metalloproteinase expression, tissue metal proteinase inhibitor manufacture, and tumor invasiveness, according to research on choriocarcinoma and pancreatic cancer[13,14]. To date, several reports on the RETN rs10401670 SNP have been made available. The SNP rs10401670 was associated with fasting plasma glucose levels and serum RETN., according to. [15] Similarly, a study of 1269 children by Ortega *et al.* revealed that the SNP rs10401670 was not only related to *RETN* levels but also to TC and low-density lipoprotein. These results revealed that the rs10401670 SNP is closely related to human metabolism[16].

CONCLUSION

RETN (rs10401670 T/C) is the only SNP that has a significant impact on cancer risk. Other SNPs, rs3745369 G/C, rs368504053 T/C, and rs74489689 A/G, were not significantly associated with BC.

Limitations of the Study:

This study has limitations, including insufficient sample size for evaluating intricate associations, lack of cause-and-effect relationships, and targeting a specific population. The control group relied on their self-reported health status and was potentially flawed. In addition, residual confounding concerns remain. Further studies should focus on larger populations and longitudinal study designs to confirm and expand the role of genetic variants in disease mechanisms.

Declarations

Availability of data and material:

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The author declare that they have no competing interests

Funding:

No funds were received to fulfill this work.

Acknowledgements:

We are truly grateful to the patients who supplied samples for our study despite their debilitating medical conditions. Al-Amal Hospital / Baghdad/Iraq, staff members made an effort to make the process of collecting samples as simple as possible. Great gratitude to the laboratories of Institute for Genetic Engineering and Biotechnology for Postgraduate Studies/ University of Baghdad for their contribution to accomplishing the practical part of this study.

Author Contribution The authors were contributed equally in conceptualized the research, collected data, participated in data analysis and write-up, editing and review.

Ethical approval

This study was performed according to the ethical rules for medical research involving human participants of the Declaration of Helsinki (1964). Before sampling, the approval of the patient or his companion was taken. The study protocol and the subject information and the consent form were reviewed and approved by Al-Amal Hospital / Medical City - Baghdad - Iraq according to document number (11581) on (19/03/2023) to get this approval.

REFERENCES

1. Abbas RAR, Aziz IH. Study comparing the oncogenic microRNA-21-5p and the CA15-3 characteristics as an effective tumor marker in breast cancer patients from Iraq. Revis Bionatura 2022; 7 (4) 10. Vol. 51, regulation. s Note: Bionatura stays neutral with regard to jurisdictional claims in …; 2019. p. 11–7.
2. Hussain AM, Ali AH, Mohammed HL. Correlation between serum and tissue markers in breast cancer Iraqi patients. Baghdad Sci J. 2022;19(3):501.
3. Salih AM, Aziz IH, Mohsin FY. Role of miRNA 199a-5p Expression in Iraqi Women with Breast Cancer. Al-Rafidain J Med Sci (ISSN 2789-3219). 2023;5(1S):S94-99.

4. Sahan KA, Aziz IH, Dawood SN, Al Qazzaz H. The role of resistin gene polymorphism in Iraqi breast cancer patients. Biomedicine. 2022;42(6):1296–300.
5. Abbas RA, Aziz IH. Role of miRNA-21-5p Gene Expression as a Tumor Marker and the Progression of Clinic-Pathological Characters in Breast Cancer Iraqi Female Patients. Iraqi J Biotechnol. 2023;22(1):8–18.
6. Liu L, Zhai W, Wang F, Yu L, Zhou F, Xiang Y, et al. Using machine learning to identify gene interaction networks associated with breast cancer. BMC Cancer. 2022;22(1):1070.
7. Dalamaga M, Sotiropoulos G, Karmaniolas K, Pelekanos N, Papadavid E, Lekka A. Serum resistin: a biomarker of breast cancer in postmenopausal women? Association with clinicopathological characteristics, tumor markers, inflammatory and metabolic parameters. Clin Biochem. 2013;46(7–8):584–90.
8. Bel'skaya L V, Loginova AI, Sarf EA. Pro-inflammatory and anti-inflammatory salivary cytokines in breast cancer: relationship with clinicopathological characteristics of the tumor. Curr Issues Mol Biol. 2022;44(10):4676–91.
9. Kolb R, Zhang W. Obesity and breast cancer: a case of inflamed adipose tissue. Cancers (Basel). 2020;12(6):1686.
10. Guo Q, Jin Y, Lin M, Zeng C, Zhang J. NF-κB signaling in therapy resistance of breast cancer: Mechanisms, approaches, and challenges. Life Sci. 2024;122684.
11. Sudan SK, Deshmukh SK, Poosarla T, Holliday NP, Dyess DL, Singh AP, et al. Resistin: An inflammatory cytokine with multi-faceted roles in cancer. Biochim Biophys Acta (BBA)-Reviews Cancer. 2020;1874(2):188419.
12. Quintero-Fabián S, Arreola R, Becerril-Villanueva E, Torres-Romero JC, Arana-Argáez V, Lara-Riegos J, et al. Role of matrix metalloproteinases in angiogenesis and cancer. Front Oncol. 2019;9:1370.
13. Slapak EJ, Duitman J, Tekin C, Bijlsma MF, Spek CA. Matrix metalloproteases in pancreatic ductal adenocarcinoma: key drivers of disease progression? Biology (Basel). 2020;9(4):80.
14. Annese T, Tamma R, Ruggieri S, Ribatti D. Angiogenesis in pancreatic cancer: pre-clinical and clinical studies. Cancers (Basel). 2019;11(3):381.
15. de Luis Román DA, Ortolá A, Delgado E, Díaz G, Torres B, López JJ. The rs10401670 variant of the resistin gene is related to circulating resistin levels, insulin resistance, and presence of type-2 diabetes mellitus in obese patients. Nutr Hosp. 2020;37(2):293–8.
16. Ortega L, Navarro P, Riestra P, Gavela-Pérez T, Soriano-Guillén L, Garcés C. Association of resistin polymorphisms with resistin levels and lipid profile in children. Mol Biol Rep. 2014;41:7659–64.