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RESEARCH

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STAT3 gene polymorphisms and susceptibility to breast cancer in the Moroccan population

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Abstract

Background Breast cancer is a complex disease due to its extremely complicated and varied etiology. It is found to be linked to improper transcription factor activation that interferes with normal breast development. Among these factors, signal transducer and activator of transcription (STAT) proteins play a crucial role in regulating gene expression and cell signaling. Specifically, STAT3, a member of the STAT family, has been found to be constitutively active in various cancer types, including breast cancer. Three *STAT3* SNPs (rs744166, rs229152, and rs4796793) were widely investigated in association with cancer diseases in many populations, yet the findings were conflicting. This study seeks to evaluate the association risk of these three SNPs with breast cancer in Moroccan women.

Materials and methods This case–control study consisted of 200 breast cancer cases and 200 age- and sex-matched healthy controls. The extraction was carried out from whole blood by the salting-out method. Genotypes were defined using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and sequence-specific primer–polymerase chain reaction (SSP–PCR) methods.

Results In the over-dominant model (GG–CC vs. GC), the rs4796793*GC genotype was linked to a higher risk of breast cancer among triple-negative cases. Additionally, a significant association has been revealed between HER2 and the mutant genotype of the two polymorphisms rs744166 and rs4796793. Moreover, the *STAT3* rs744166*AG genotype was less common in cases with late-stage (grade III) disease.

Conclusion These findings suggest that *STAT3* polymorphisms are associated with triple-negative breast cancer and HER2+ type; the top two lethal breast cancer in Moroccans.

Keywords *STAT3* gene, rs744166, rs229152, rs4796793, Breast cancer risk, Moroccan population

Introduction

Breast cancer is one of the most common forms of cancer among women globally. In 2020, there were roughly 2.3 million new cases of breast cancer reported worldwide which accounted for around 24.5% of all new cancer cases diagnosed in women [1]. In Morocco, breast cancer is a major public health concern, being the most commonly diagnosed cancer among women in the country. According to the WHO, it represents almost 40% of all cancers diagnosed in women in 2020 [1].

Breast cancer is a complex pathology characterized by multifactorial mechanisms, including genetic

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predisposition and environmental factors. Growing evidence suggests that the immune system plays a critical role in cancer susceptibility and development. Previous studies have shown that genetic variations in genes involved in regulating immunity, such as *STAT3*, may play a significant role in breast cancer susceptibility [2, 3].

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor encoded by the *STAT3* gene [4]. STAT3 activity regulates a plethora of genes implicated in numerous normal cellular processes including proliferation, differentiation, cell proliferation, apoptosis, inflammation, and immune responses [5]. STAT3 plays a fundamental role in normal mammary gland development and is also implicated in mammary oncogenesis [6, 7].

Aberrant STAT3 activation has been strongly associated with tumor progression by regulating gene expression involved in angiogenesis and invasion [5]. This anomalous activation of STAT3 has been shown to be present in a variety of human malignant tumors, including breast cancer [8]. This transcription factor regulates the expression of many genes, which promote tumor progression. These include the genes that encode Bcl-xL, cyclin D1 and D2, c-MYC, and MCL1, eventually leading to cellular transformation by increasing proliferation and slowing-down apoptosis [9]. Recent genetic studies have demonstrated that genetic variants in the *STAT3* gene influence numerous human malignancies' susceptibility, development, and therapy outcomes [10–13].

STAT3 was discovered in 1994 for the first time as a DNA-binding protein in response to interleukin-6 and epidermal growth factor [14]. To date, there are seven members of this protein family: STAT1, 2, 3, 4, 5A, 5B, and 6 [8]. Most immune regulatory systems including tumor cell identification and escapement are mediated by the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway [15]. Janus kinases (JAKs) are activated by cytokines including interleukin-6 (IL-6) and interleukin-10 (IL-10), hormones, and growth factors as well as oncogenic proteins, such as Src [16] and Ras [17]. Upon activation, JAKs phosphorylate STAT3, which forms homodimers and translocate to the nucleus to activate the transcription of specific genes that drive cancer progression [18, 19]. Recent clinical and preclinical data indicate the involvement of overexpressed and constitutively activated STAT3 in the progression, proliferation, metastasis, and chemoresistance of breast cancer [20]. It has been proven that STAT3, in particular, performs a crucial function in the pathological process of human breast cancer as well as normal mammary gland development [6, 7]. STAT3 is implicated in the post-lactational regression and apoptosis of the mammary gland [7]. STAT3 has been mentioned in several studies to be associated with oncogenesis via several mechanisms, including apoptosis inhibition, cell proliferation promotion, angiogenesis induction, and immune response suppression [21].

Three *STAT3* polymorphisms were investigated in the current study, including rs744166 (in intron 2), rs229152 (in intron 11), and rs4796793 (in the promoter). The three polymorphisms have been studied previously in relation to several neoplasms; however, the findings were conflicting [22].

The aim of the present study was to investigate the association of these three *STAT3* polymorphisms with breast cancer risk in Moroccan women.

Materials and methods

Study population

Four hundred unrelated Moroccan women were enrolled in this case–control study (200 breast cancer cases and 200 age- and sex-matched healthy controls). Patients have been recruited during two years from 2017 to 2019. All cases were confirmed histopathologically and were receiving medical treatment at the Regional Center of Oncology and Radiotherapy; Hospital Hassan II, Agadir. Blood donor women with no personal or family cancer history were also recruited as the control group. Cases with unclear diagnostic and controls with personal or familial breast cancer history were excluded from the study. We extracted all patients' information including age, sex, and menopausal status from clinical records. Tumor description including SBR grading, ER receptor, HER2 receptor, tumor histology, PR receptor, and IHC subtypes were also assessed in this study. All subjects gave informed consent before participating in the study. The study was conducted in accordance with the Helsinki Declaration, and the protocol was approved by the Ethics Committee of Cadi Ayyad University Hospital Center (CHU) Mohammed VI, Marrakech, Morocco.

Genotyping

Samples were collected from venous blood in an EDTA tube, and the common salting-out method described by Miller et al. was used to isolate genomic DNA [23].

The genotyping of rs744166 and rs229152 polymorphisms was carried out using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. The amplified PCR products were processed with restriction enzyme overnight (rs744166 by *HindIII* and rs229152 by *HpaII*), and then, the products were analyzed by electrophoresis in 2–4% agarose gel.

Genotyping for rs4796793 was performed using polymerase chain reaction with sequence-specific primer–polymerase chain reaction (SSP–PCR) method

as described by Wang et al. [24]. A fragment of 502 bp results from a first PCR performed using forward (F) and common reverse (R) primers. The second one was done using the common reverse (R) and specific forward C (F1) primers for the wild-type allele and the common reverse (R) and specific forward G (F2) primers for the amplification of the mutant allele. The two alleles' fragments result in a fragment of approximately 316 bp. Table 1 provides further details about these assays.

Approximately 10% of all samples were genotyped in duplicate to verify results.

Statistical analysis

To assess whether the distributions of *STAT3* genotypes were in Hardy–Weinberg equilibrium, we used SNPStats software [27]. IBM SPSS Statistics (version 25.0) [28] was used to evaluate genotypic and allelic frequencies. The association between *STAT3* genetic variants and breast cancer risk was also assessed using SPSS software using odds ratios (ORs) with 95% confidence intervals adjusted for age and menopausal status. The link between *STAT3* polymorphisms' haplotypic frequencies and the risk of breast cancer was estimated by SNPStats software. Both SNPstats and SPSS software were used to assess the association between each *STAT3* genetic variant and clinical characteristics. Linkage disequilibrium (LD) was assessed by Haploview software (version 4.2) [29].

Results

Detailed information for the three SNPs is abstracted in Table 2, including gene location, minor allele frequency (MAF), functional annotations, and HWE p-value. Hardy–Weinberg equilibrium (HWE) was performed within the control and case groups (Table 2). Both the cases' and controls' genotypic frequencies were within the HWE ($p > 0.05$).

Detailing clinicopathological characteristics of breast cancer patients are presented in Table 3 [30].

Figure 1 depicts an ethidium bromide-stained agarose gel illustrating the three SNPs. Images (A), (C), and (B), respectively, show PCR–RFLP results for the STAT3 polymorphism rs744166 and rs2293152 and SSP–PCR results for the STAT3 polymorphism rs476793.

The genotype and allele frequencies of each SNP are listed in Table 4. The minor allele frequencies of rs744166, rs2293152, and rs4796793 were about 51%, 32%, and 32% in breast cancer cases and 51%, 37%, and 34% in controls, respectively. According to statistical analysis, adjusted for age and menopausal status, there was no statistically significant association between the risk of breast cancer and the *STAT3* variants (Table 4).

Table 1 Primers and PCR conditions used for the three studied SNPs

SNP	Primers		PCR conditions	PCR product (bp)	Reference
rs744166	Forward	GCTGTAATGTCTTGAGGGAATCAAGC	95 °C—5 min.; 35 cycles (94 °C—0.5 min., 58 °C—1 min. and 72 °C—1 min.) and 72 °C—5 min	144	[25]
	Reverse	TATTCAGATGGCGGTCACATGC			
rs2293152	Forward	TCCCCTGTGATTCAGATCCC	95 °C—5 min.; 35 cycles (94 °C—0.5 min., 55 °C—1 min. and 72 °C—1 min.) and 72 °C—5 min	214	[26]
	Reverse	CATTCCCACATCTCTGCTCC			
rs4796793	Forward primer (F)	TCTGGTAGACACAGCTCAGTATGG	95 °C—5 min.; 35 cycles (94 °C—1 min. (65 °C for the first PCR and 66 °C for the second PCR)—1 min. and 72 °C—1 min.) and 72 °C—5 min	First PCR: 502	[24]
	Common reverse primer (R)	CCATAGTCGCAGAGGTAGATTTTA			
	Specific forward primer C (F1)	TGTTTAGTGATTTACTGCTTACAA AGG			
	Specific forward primer G (F2)	TGTTTAGTGATTTACTGCTTACAA AGC		Second PCR: 316	

Table 2 Description of the three SNPs

SNP	Location	Variation	MAF % case/control	HWE *p* value case/control
rs744166 A>G	chr17:42362183 (GRCh38.p13)	Intron	51/51	0.89/0.26
rs2293152 G>C	chr17:42329511 (GRCh38.p13)	Intron	32/37	0.42/0.88
rs4796793 G>C	chr17:42390192 (GRCh38.p13)	Upstream transcript variant	32/34	1/0.75

MAF minor allele frequency

Table 3 Clinicopathological characteristics of BC cases

Clinicopathological characteristics	Cases *n* (%) *N* = 200	Controls *n* (%) *N* = 200	*P* value
Age (mean ± SD)	48.20 ± 10.52	48.21 ± 10.46	1
Menopause			
Pre	130 (65)	136 (68)	0.53
Post	70 (35)	64 (32)	
Tumor histology			
IDC	149 (84.66)	–	–
ILC	16 (9.09)		
Medulary	1 (0.57)		
Other	10 (5.68)		
SBR grading			
I	9 (5.14)	–	–
II	102 (58.29)		
III	64 (36.57)		
Estrogen receptor (ER)			
Pos	143 (78.57)	–	–
Neg	39 (21.43)		
Progesterone receptor (PR)			
Pos	110 (68.32)	–	–
Neg	51 (31.68)		
Human epidermal growth factor 2 (HER2) receptor			
Pos	46 (29.11)	–	–
Neg	112 (70.89)		
Immuno-histochemical (IHC) subtypes			
Luminal A	85 (54.49)	–	–
Luminal B	35 (22.44)		
Triple neg	26 (16.66)		
HER2/neu	10 (6.41)		

IDC invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *Neg* negative, *Pos* positive, *Luminal A* ER+ and/or PR+ ER−/PR+ with HER2−, *Luminal B* ER+ and/or PR+ ER−/PR+ with HER2+, *Triple-negative* ER−, PR−, HER2−, *HER2/neu* ER−, PR− with HER2+

However, the results suggest a potential link, evaluated as an OR adjusted for menopausal status and age, between *STAT3* polymorphisms (rs4796793 and rs744166) and clinical characteristics of breast cancer (Table 5). However, statistical findings show no significant link between rs2293152 with clinical characteristics. The rs744166*GG genotype was found to be more common in HER2-positive cases (OR 2.97 (1.08–8.13); $P = 0.0323$), while the rs744166*AG genotype was associated with breast cancer among grade 3 cases (OR 2.23 (1.05–4.71); $P = 0.0373$). Additionally, the rs4796793*CC genotype was associated with breast cancer within HER2-positive cases (OR 0.30 (0.09–0.95); $P = 0.0281$). In the over-dominant model (GG–CC vs GC), the genotype rs4796793*GC showed an association with breast cancer within triple-negative cases (OR 2.43 (1.01–5.82) $p = 0.0397$).

The haplotype ACG was the most prevalent (frequency 0.2716), followed by the GGC haplotype (0.2452). Moreover, Table 6 shows the generated haplotypes and their association with breast cancer risk. According to the results, null association was observed between the generated haplotypes and breast cancer risk.

Based on the LD calculation among the three SNPs, no LD between these polymorphisms was found (Fig. 2).

Discussion

STAT3 gene is located on chromosome 17q21.2 [31]. STAT3 protein is a member of a family of seven transcription factors that are a component of the JAK-STAT signaling pathway which underlies the signal transduction mechanism of many cytokine receptors [32]. STAT3 can upregulate the transcription of genes implicated in immunological and anti-apoptotic processes, as well as genes involved in cell survival and proliferation [33]. STAT3 is one of the STAT protein family's seven members and is highly activated in many cancers, including breast cancer, prostate hepatocellular carcinoma, lymphoma, non-small cell lung cancer, and multiple myeloma [34].

This transcription factor has dual crucial roles as signal transduction proteins from extracellular stimuli frequently activated in cancer cells and as nuclear transcription factors that regulate the expression of a diverse set of genes, contributing to cancer progression [35, 36]. Among the seven *STAT* members, *STAT3* is the most important one for cancer progression [9, 37]. Although the main role of *STAT3* in normal mammary gland development has been studied as an inducer of apoptosis and cell elimination during involution, abnormal STAT3 activation may also contribute to breast cancer formation and progression. Studies have revealed that STAT3 is constitutively activated at a percentage that varies between 35 and 60% in human breast cancers and is associated with an increased risk of metastasis, high tumor grade, and high risk of recurrence [38–40]. *STAT3* is a polymorphic gene, and numerous researches have studied how *STAT3* single-nucleotide polymorphisms (SNPs) affect various populations' risk of developing cancer [22]. It has been proposed that *STAT3* SNPs may affect STAT3 activation and expression after stimulation and increase the chances of developing inflammatory and malignant disorders [22].

According to the present study, the presence of rs4796793*GC genotype under the over-dominant model (GG–CC vs GC) was associated with an increased risk of breast cancer within triple-negative cases (OR 2.43 (1.01–5.82); $p = 0.044$). In our dataset, triple-negative breast cancer cases account for 16.66% of all subtypes [30]. Triple-negative breast cancer affects young women,

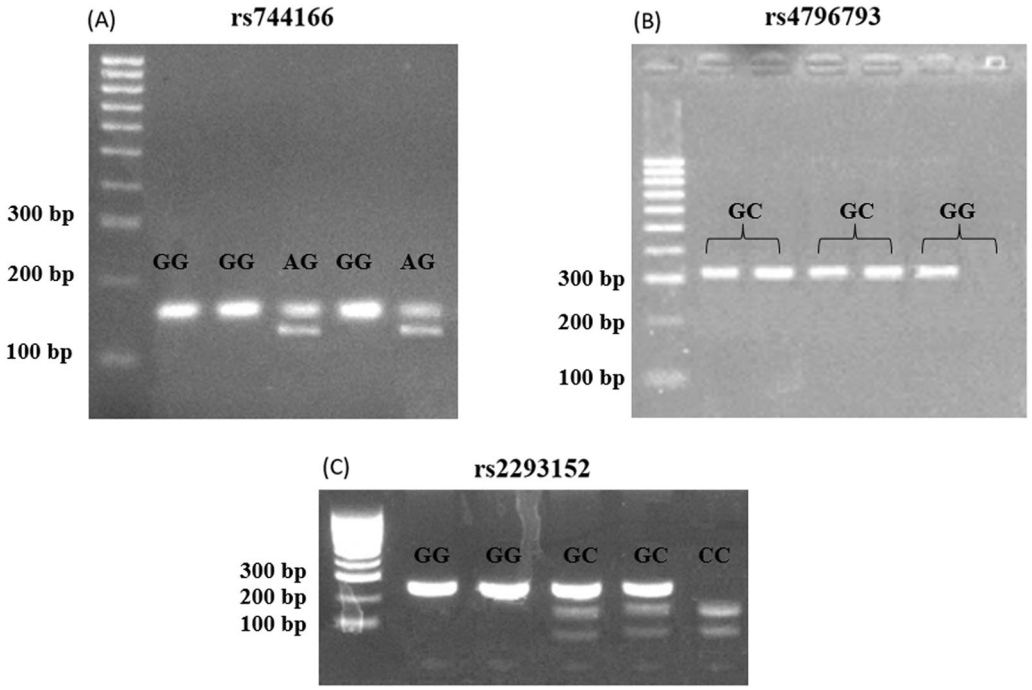

Fig. 1 Ethidium bromide-stained agarose gel showing the three SNPs

Table 4 *STAT3* polymorphisms' genotype and allele frequencies in breast cancer cases and healthy controls

Genotype	Case *n* (%)	Control *n* (%)	OR (95% CI)[a]	*P* value
rs744166				
AA^{wt}	48 (24)	52 (26)	1	
AG	99 (49.5)	92 (46)	1.15 (0.72–1.84)	0.78
GG	53 (26.5)	56 (26)	0.99 (0.58–1.71)	
A^{wt}	195 (49)	196 (49)		
G	205 (51)	204 (51)		
rs2293152				
GG^{wt}	89 (44.50)	80 (40)	1	
GC	93 (46.50)	92 (46)	0.92 (0.61–1.41)	0.28
CC	18 (9)	28 (14)	0.59 (0.30–1.14)	
G^{wt}	271 (68)	252 (63)		
C	129 (32)	148 (37)		
rs4796793				
GG^{wt}	93 (46.5)	89 (44.5)	1	
GC	87 (43.5)	87 (43.5)	0.95 (0.63–1.45)	0.75
CC	20 (10)	24 (12)	0.77 (0.40–1.51)	
G^{wt}	273 (68)	265 (66)		
C	127 (32)	135 (34)		

[a] Adjusted for age and menopausal status

and it represents around 15% of all breast cancer cases across populations and is more prevalent in women of African and Hispanic ancestry [41]. In another Moroccan study, the triple-negative breast cancer rate was 16.67%, which is consistent with our dataset [42]. But an epidemiological Moroccan study reported a rate of 20.26% for triple-negative breast cancer among 1559 cases [43]. Although STAT3 is upregulated in all subtypes of breast cancer, it is more frequently linked to triple-negative tumors, in which HER2 is not overexpressed and does not express estrogen (ER) or progesterone receptors (PR) [44]. Indeed, *STAT3* rs4796793 polymorphisms could be used as a possible marker for detecting malignant triple-negative breast cancer [45]. Additionally, a study using an in silico method discovered that the STAT3 protein was found to be excessively expressed in triple-negative breast cancer and negatively correlated with lymph node implication and breast cancer clinical stage [3]. Triple negatives are more likely to have MYC and STAT3 abnormal expressions. These two molecules enhance tumor anti-apoptotic activity, metastasis, vascularity, and histological grade [46]. Our findings also showed an association between the mutant genotype of the two *STAT3* polymorphisms rs744166 and rs4796793 and HER2. In our study, 29.11% of all subtypes are HER2-positive cases. However, its frequency matched the positive frequency of HER2, which ranges between 25 and 30% across several investigations [40, 47, 48]. According to a Moroccan study, the HER2 protein is overexpressed in 29.17% of tumors in breast cancer cases [43]. Both triple-negative breast cancer and HER2+ have been regarded as

Table 5 Correlation of *STAT3* polymorphisms with clinical characteristics of cases with breast cancer

	OR (95% CI)						
	Menopausal status	**Tumor histology**	**SBR grading**	**Estrogen receptor (ER)**	**Progesterone receptor (PR)**	**Human epidermal growth factor 2 (HER2)**	**Immuno-histochemical (IHC) subtypes**
rs744166							
AA	1	1	1	1	1	1	1
AG	2.20 (0.39–12.33)	1.03 (0.38–2.80)	**2.23 (1.05–4.71)**	0.94 (0.39–2.30)	0.74 (0.33–1.65)	1.87 (0.83–4.23)	2.10 (0.64–6.83)
GG	0.76 (0.33–1.72)	1.02 (0.32–3.19)	2.01 (0.84–4.83)	1.01 (0.36–2.80)	0.61 (0.23–1.57)	**2.97 (1.08–8.13)**	1.42 (0.37–5.54)
	p:0.46	IDC vs others p:1	Grade I and II vs III **p:0.0373**	p:0.99	p:0.57	**p:0.0323**	Triple negative vs others p:0.4
AA–GG	1	1	1	1	1	1	1
AG	1.24 (0.35–5.03)	1.02 (0.45–2.32)	1.60 (0.85–3.01)	0.94 (0.45–1.94)	0.94 (0.48–1.83)	1.13 (0.57–2.25)	1.72 (0.72–4.09)
	p:0.76	p:0.96	p:0.14	p:0.87	p:0.86	p:0.73	p:0.21
rs2293152							
GG	1	1	1	1	1	1	1
GC	2.50 (0.55–11.83)	1.20 (0.52–2.75)	1.05 (0.54–2.03)	1.24 (0.58–2.67)	0.71 (0.35–1.42)	1.57 (0.77–3.23)	2.05 (0.80–5.23)
CC	0.54 (0.50–6.21)	7.99 (0.45–140.47)	0.62 (0.21–1.82)	0.83 (0.21–3.34)	0.42 (0.11–1.66)	3.40 (0.70–16.48)	2.07 (0.47–9.09)
	p:0.31	p:0.065	p:0.63	p:0.77	p:0.35	p:0.17	p:0.28
GG–CC	1	1	1	1	1	1	1
GC	2.83 (0.67–11.88)	1.94 (0.41–2.14)	1.14 (0.61–2.14)	1.28 (0.62–2.66)	0.80 (0.41–1.58)	1.34 (0.67–2.69)	1.76 (0.75–4.15)
	p:0.15	p:0.88	p:0.68	p:0.51	p:0.52	p:0.41	p:0.19
rs4796793							
GG	1	1	1	1	1	1	1
GC	1.42 (0.32–6.36)	0.47 (0.19–1.20)	0.77 (0.39–1.51)	1.93 (0.87–4.30)	1.48 (0.72–3.02)	1.56 (0.72–3.38)	1.96 (0.81–4.75)
CC	0.47 (0.40–4.94)	0.37 (0.10–1.42)	0.59 (0.20–1.73)	1.92 (0.56–6.58)	1.11 (0.34–3.67)	**0.30 (0.09–0.95)**	0.21 (0.01–3.88)
	p:0.64	p:0.19	p:0.57	p:0.23	p:0.56	**p:0.0281**	p:0.09
GG–CC	1	1	1	1	1	1	1
GC	1.65 (0.40–6.88)	0.60 (0.26–1.38)	0.86 (0.46–1.62)	1.67 (0.80–3.49)	1.45 (0.73–2.85)	1.98 (0.95–4.12)	**2.43 (1.01–5.82)**
	p:0.49	p:0.23	p:0.64	p:0.17	p:0.29	p:0.062	**p:0.0397**

Significant results are mentioned in bold

Table 6 Haplotype constructions of *STAT3* polymorphisms

	rs744166	**rs2293152**	**rs4796793**	**Frequencies**	**OR (95% CI)**	***P* value**
1	A	C	G	0.2716	1	–
2	G	G	C	0.2452	0.99 (0.67–1.48)	0.97
3	G	G	G	0.2048	0.69 (0.44–1.08)	0.1
4	A	G	G	0.1763	0.84 (0.53–1.34)	0.47
5	G	C	C	0.0415	1.09 (0.43–2.75)	0.85
6	A	G	C	0.0274	0.52 (0.18–1.52)	0.23
7	G	C	G	0.0197	1.49 (0.35–6.31)	0.59
8	A	C	C	0.0134	0.96 (0.18–5.41)	0.96

the top two lethal subtypes of breast cancer due to their poor prognostic features [49]. Additionally, our findings are the first to suggest that the *STAT3* rs744166*AG genotype was less occurred in cases with late-stage (grade III) cancer. The three *STAT3* polymorphisms' allele and genotype frequencies were not significantly associated with the menopausal state, tumor histology, estrogen receptor, or progesterone receptor.

Three *STAT3* polymorphisms—rs744166, rs2293152, and rs4796793—were investigated in this study. The

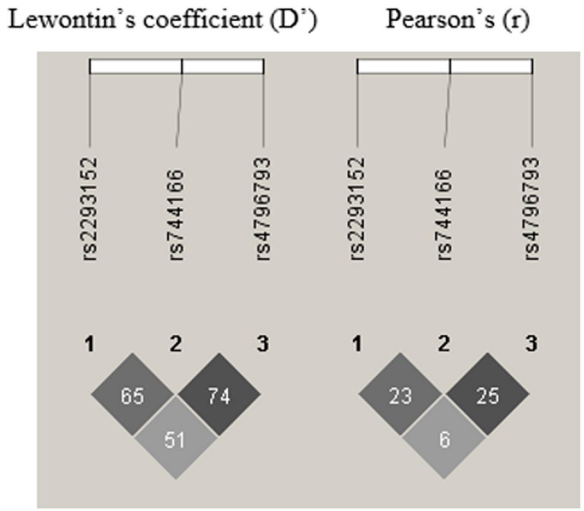

Fig. 2 Linkage disequilibrium plot of three SNPs within *STAT3*. Lewontin's coefficient (*D'*) and Pearson's (*r*) statistics were used to calculate LD

three polymorphisms have been previously investigated with regard to different cancers, but the results were inconsistent [12, 50–52]. Our results show no statistically significant link between any of the three variants and breast cancer in Moroccan women. Similar findings were reached in other studies, for instance, a case–control study of German breast cancer cases, which revealed no statistically significant link between rs2293152 and breast cancer [12]. Regarding other cancer types, two studies found no statistically significant link between rs2293152 polymorphism and gastric [52] or lung cancer [50]. Contrary, Yan et al. found that the *STAT3* rs744166 polymorphisms reduced significantly the incidence of cancers [22]. *STAT3* rs4796793 was associated with increased susceptibility to lung cancer [53] and decreased risk of breast cancer [13]. Furthermore, the *STAT3* rs2293152 polymorphism has been linked to a higher risk of basal cell carcinoma [54].

This study reported the association of *STAT3* polymorphisms with triple-negative breast cancer and HER2+, and this finding could help to better understand the molecular mechanisms underlying breast cancer in the Moroccan population and to identify people at high risk of developing this disease. Further studies are needed to understand the etiology of breast cancer and to detect the involvement of immunity genes in breast cancer outcome and prognostics for more appropriate medical treatment and long-term survival.

Conclusion

In summary, triple-negative cases with the rs4796793*GC genotype (GG–CC vs GC) are at an elevated risk of developing breast cancer. Our research also revealed an association between HER2 and the mutant genotype of the two *STAT3* polymorphisms, rs744166 and rs4796793. Furthermore, the *STAT3* rs744166*AG genotype was found to be less common in cases with late-stage (grade III) disease.

Abbreviations

Bcl-xL	B-cell lymphoma-extra large
CI	Confidence interval
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EM	Expectation maximization
ER	Estrogen receptors
JAK	Janus kinase
HER2	Human epidermal growth factor 2
HWE	Hardy–Weinberg equilibrium
IHC	Immuno-histochemical subtypes
LD	Linkage disequilibrium
MAF	Minor allele frequency
MCL1	Myeloid cell leukemia sequence 1
OR	Odds ratio
PCR	Polymerase chain reaction
PCR–RFLP	Polymerase chain reaction–restriction fragment length polymorphism
PR	Progesterone receptors
SBR	Scarff–Bloom–Richardson
SNP	Single-nucleotide polymorphism
SSP–PCR	Sequence-specific primer–polymerase chain reaction
STAT	Signal transducer and activator of transcription

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Author contributions

NI contributed to the conceptualization, methodology, software, data curation, and writing—original draft preparation. SEA contributed to the conceptualization, methodology, software, writing—reviewing, and editing. EHI contributed to the conceptualization, writing—reviewing, and supervision. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the results are included within the article. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

Declarations

Ethics approval and consent to participate

All subjects gave informed consent before participating in the study. The study was conducted in accordance with the Helsinki Declaration, and the protocol was approved by the Ethics Committee of Cadi Ayyad University Hospital Center (CHU) Mohammed VI, Marrakech, Morocco.

Consent for publication
Written informed consent was obtained from all patients and controls.

Competing interests
The authors declare that they have no competing interests.

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