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ORIGINAL ARTICLE

Association of *TNFRSF1A* and *IFNLR1* Gene Polymorphisms with the Risk of Developing Breast Cancer and Clinical Pathologic Features

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Abstract

Several genes have been associated with breast cancer (BC) susceptibility. The tumor necrosis factor receptor superfamily, member 1A (*TNFRSF1A*), and interferon lambda receptor 1 (*IFNLR1*) genes encode receptors that mediate the action of inflammatory cytokines. Previous studies have demonstrated the association of the variants rs1800693 (*TNFRSF1A*) and rs4649203 (*IFNLR1*) with some inflammatory diseases. The present study aimed to verify a possible association of these variants with BC, its clinical pathologic features, as well as epidemiological data in a Brazilian population. A total of 243 patients and 294 individuals without history of BC were genotyped for these polymorphisms through TaqMan® SNP genotyping assays by qPCR. For the *TNFRSF1A* gene, no significant results were found. For *IFNLR1*, the AA genotype ($p=0.008$) and the A allele ($p=0.02$) were significantly associated with a lower risk of developing BC. When analyzing the age, it was observed that each increase of one year contributes to the development of BC ($p<0.001$). Also, the smoking habit ($p<0.001$) and body mass index ($p=0.018$) increase the risk of disease development. Analyzing progesterone receptor factor an association was found with the AA genotype of the *IFNLR1* ($p=0.02$). The findings suggest that polymorphism in the immune-related *IFNLR1* gene contribute to BC susceptibility in a Brazilian population. These findings can contribute to the further understanding of the role this gene and pathways in BC development.

Keywords Interleukin-28 receptor alpha · Tumor necrosis factor receptor superfamily · Member 1A · Breast cancer · Smoking habit · Progesterone receptor factor

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Introduction

Breast cancer (BC) is the most commonly diagnosed and the most deadly cancer in women worldwide (The Union International Cancer Control 2018). The GLOBOCAN estimates approximately 2 million (24%) new cases and 622,000 deaths of women of BC worldwide in 2018 (representing the fifth cause of death in the world) (Bray et al. 2018). BC is the main type of cancer among Brazilian women, according to the Instituto Nacional de Câncer. In 2018 approximately 17,000 BC deaths (16.4%) were registered in Brazil (Instituto Nacional de Câncer José Alencar Gomes da Silva 2019) and 66,280 (29.7%) new cases of BC per year are predicted to occur in the next triennium. This value corresponds to an estimated risk of 61.61 new cases per 100 thousand women.

The tumor progression is controlled by a complex interplay between the host immune response and the cancer cells (Gonzalez et al. 2018). However, cancer cells tend to accumulate mutations, which can acquire specific phenotypes, such as the ability to avoid and resist the host immune response (de Kruijf et al. 2010; Parcesepe et al. 2016). Previous studies have shown that chronic inflammation in the vicinity of the tumor microenvironment can promote the growth as well as the progression of BC (Ben-Baruch 2003; Lu et al. 2006; Elinav et al. 2013; Iyengar et al. 2015). Inflammatory responses play decisive roles at different stages of tumor development, including pathogenesis, invasion, and metastasis, and therefore, inflammatory cytokines are critical components of tumor progression. Cytokines activate their immunomodulatory effects through their interaction with specific receptors. Thus, genetic polymorphisms in genes responsible for coding these receptors may result in altered expression and/or structural changes that may affect cytokine biological activity (Dhiman et al. 2010; Moudgil and Choubey 2011).

Genetic variants in inflammation-related cytokine receptors genes tumor necrosis factor receptor superfamily, member 1A (*TNFRSF1A*), and interferon lambda (INF-λ) receptor 1 *(IFNLR1*) also known as interleukin 28 receptor, alpha (*IL28RA*) have been associated with inflammatory diseases (De Jager et al. 2009; Strange et al. 2010b; Gregory et al. 2012; Lopez de Lapuente et al. 2012; Li et al. 2013; Park et al. 2013; Yang et al. 2013; Xu et al. 2014; Hoffjan et al. 2015; Ma et al. 2017; Javor et al. 2018; Shao et al. 2018; Watts et al. 2019; Dashti et al. 2020). The interaction of these receptors with their ligands can regulate inflammation and increase the invasive activity and metastatic potential of tumor cells as reported by a few studies (Xu et al. 2014; Hengeveld and Kersten 2015; Jiang et al. 2017). However, currently there are few studies relating these genes to susceptibility to BC.

TNFR1 is a major receptor for tumor necrosis factor alpha (TNF-α) and belongs to a superfamily of receptors for TNF. TNF-α is produced by neoplastic cells or cells in the tumor microenvironment and can acts as an endogenous tumor promoter. In normal breast tissue, TNF-α regulates cell proliferation through its pro-apoptotic effects, but in BC, the inhibition of the apoptotic pathway and the enhancement of the survival and proliferation effects contribute to tumor cell proliferation (García-Tuñón et al. 2006).

As one of the receptors for TNF-α, TNFR1 can activate NF-Kappaβ, mediate apoptosis, and regulate inflammation (Xu et al. 2014). Genetic variants in the *TNFRSF1A* gene may be responsible for causing structural and/or functional changes in the receptor, which in turn may result in an imbalance in TNF cytokine action (Dhiman et al. 2010). A variant in this gene (rs1800693) has been identified and functional studies showed that this variant leads to the production of a novel isoform of TNFR1 (Comabella et al. 2013; Kulakova et al. 2018). This isoform lacks the C-terminal portion, including the transmembrane domain and the intracellular regions that are essential for its appropriate cellular localization (Gregory et al. 2012; Comabella et al. 2013). The rs1800693 is located at intron 6 of the *TNFRSF1A* gene and is believed to influence exon 6 splicing. In an in vitro splicing assay, the C allele resulted in a transcript that does not have exon 6, Δ6-TNFR1. When analyzing Δ6-TNFR1-mediated signaling, it was observed that the absence of the death domain affected the signal transduction that induces apoptosis (Gregory et al. 2012).

The protein encoded by *IFNLR1* gene belongs to the class II cytokine receptor family and forms a receptor complex with interleukin 10 receptor, beta (IL10RB). This complex has been shown to interact with type III interferons (Sheppard et al. 2003; Cheng et al. 2015). The IL28RA interacts with INF-λ which has immunomodulatory properties, including the process of cellular apoptosis, important in the cancer scenario (Numasaki et al. 2007; Li et al. 2009). The cellular signaling of IFN-λ, through its receptor complex, induces the activation of the JAK/STAT pathway (Janus Kinase/Signal Transducers and Transcription Activators). Ultimately, this pathway results in the induction of responsive elements stimulated by IFN and initiates the transcription of target genes (de Groen et al. 2014). In the context of cancer, these IFN-λ-activated genes are involved in inducing antiproliferative responses on tumor cells (Li et al. 2009). Alterations in the gene encoding one of the IFN-λ receptor subunits, IL28RA, may disrupt the pathway this cytokine acts on. One of the changes reported is variant rs4649203, located in the 3′ untranslated region (3′UTR) of the *IFNLR1* gene which may affect IL28RA receptor expression (Griffiths et al. 2015; Cai et al. 2015).

Thus, both genes code for receptors for key cytokines in inflammatory pathways. The tumor necrosis factor (TNF) pathway has long been implicated in inflammatory diseases and cancer and is one of the essential pro-inflammatory cytokines found in the tumor microenvironment of breast cancer patients. Concerning the receptor gene, SNP rs1800693 had previously been associated with BC in a Chinese population (Xu et al. 2014), but no other studies followed up this association or in other populations.

IL28RA receptor is also a key receptor in the Interferon (IFN)-lambdas pathways and meta-analysis of the prognostic value of *IFNLR1* gene in various cancers found that the expression of IL28RA was indeed related to the cancer prognosis in certain cancers (Yang et al. 2010a). The variant rs4649203 in this gene has been implicated in inflammatory diseases (Strange et al. 2010a; Lopez de Lapuente et al. 2012; Li et al. 2013; Yang et al. 2013) and is believed to have impact on several inflammatory pathways that are important in cancer development. Beyond the Chinese study, no other study investigated the association of these variants with any

type of cancer. Thus, it was hypothesized that those variants could also contribute to BC susceptibility.

Considering the role of these receptors in inflammatory responses and potentially to tumorigenesis, in this case–control study was investigated whether variants in the immune modulating cytokine receptors genes *TNFRSF1A* (rs1800693) and *IFNLR1* (rs4649203) contribute to the susceptibility to BC. Furthermore, was investigated how some epidemiological factors and clinical features may be associated with BC development and the genetic variants analyzed.

Materials and Methods

Patients and Controls

This study involved a total of 537 women (20 to 96 years of age): 243 BC patients (27 to 96 years of age) and 294 controls (20 to 94 years of age) without documented history of cancer, from the state of Santa Catarina, Southern Brazil (covering an area of approximately 95.000 km2). Epidemiological data, such as age (years), smoking habits, and body mass index (BMI), were collected. All patients had histopathologically confirmed primary BC diagnosis (TNM stages I–III). The clinical features of the BC patients analyzed included lymph node metastasis, the estrogen receptor (ER), progesterone receptor (PGR), and human epidermal growth factor receptor 2 (C-erbB-2). Samples were obtained at the Polydoro Ernani de São Thiago University Hospital (HU/UFSC) following the ethics standards on human experimentation. Only the women who agreed to participate and gave their written informed consent were included in this study. This study was approved by the local ethics committees (protocol nº 922.167).

Genotyping of Variants

DNA was extracted from peripheral blood samples by salting-out method (Miller et al. 1988). The variants rs1800693 in *TNFRSF1A* and rs4649203 in *IFNLR1* were genotyped by pre-designed TaqMan genotyping assay (Thermo Fisher Scientific, cat nr 4,351,379; C_2645714_10 and cat nr 4,351,379; C_27915464 10, respectively) following the manufacturer recommendations.

Statistical Analysis

The Hardy–Weinberg equilibrium (HWE) for the distribution of the two variants was analyzed using the goodness-of-fit χ^2 test by comparing the observed genotype frequencies with the expected frequencies in both groups the cases and controls (GENEPOP by Rousset and Raymond 1995). To analyze the association of the genetic factors with BC development, a univariate logistic regression (Model 1) was performed, with unadjusted odds ratios (OR) and 95% confidence intervals (CI) estimated. Three epidemiological characteristics were included (age, BMI, and smoking

habit) for univariate logistic regression as confounders. Significant associated variables with the outcome were selected and included in a multivariate logistic regression (Model 2). Poisson regression analysis was performed to analyze the genetic variables associated with the pathological characteristics (ER; PGR; C-erbB-2; LN involvement). The factors associated with univariate analysis were identified, with estimation of unadjusted prevalence ratios (PR) and 95% confidence intervals (CI) using a robust variance. Next, a multivariate Poisson regression was performed adjusting for the significant variables. Univariate analyses were called Model 1 and multivariate analyses were called Model 2 (Barros and Hirakata 2003). Statistical analyses were performed using the SPSS 20.0 software and a p-value of <0.05 was considered statistically significant. In addition, the Pearson Chi-square test was used to analyze the association of the genetic variants with BC through the genetic models dominant, recessive, and codominant. For this analysis, the WinPepi 11.65 software (by Joseph H Abramson 2016) was used and a p-value of <0.05 was also considered statistically significant.

Results

Epidemiological Features and Genotypic and Allelic Frequencies

The mean age was 53.75 ± 13.61 years in patients (27 to 96 years of age) and 45.00 ± 16.14 years in controls (20 to 94 years of age). Approximately 40% (n = 94) of patients and 11% (n = 32) of controls were smokers. In the BC group the mean BMI was 27.05 ± 5.29 (15.06 to 54.08) and in the control group it was 25.79 ± 5.70 (16.88 to 47.48).

The genotypic and allelic frequencies in BC patients (cases) and controls are described in Table 1. Frequency distributions for polymorphic sites in cases and controls were not in accordance with the HWE ($p < 0.001$). However, the absence of HWE does not affect the results found, since the two groups (case and control) for both loci share the deviation due to excess heterozygotes. This deviation can be explained by chance or even by the samples selection, women with and without cancer, with preference for a similar age group. For *TNFRSF1A* polymorphism the C allele had a frequency of 0.45 and for the T allele it was 0.55 in both controls and cases. For the *IFNLR1* polymorphism the A allele presented a frequency of 0.54 in the control group and 0.49 in the cases group, and in the G allele it was 0.46 in the controls and 0.51 in the cases.

Associations Between Epidemiological Data and Breast Cancer Development

Analyzing the epidemiological data individually, some associations were found. Considering the age, it was observed that an increase of one year contributes to an increase of 0.03 in the risk for BC ($p < 0.001$, OR = 1.03, 95% CI: 1.02– 1.05) (Table 1). The same pattern was observed with BMI, where an increase of one unit was associated with an increment of 0.04 in the risk of developing BC ($p = 0.018$,

Table 1 Genotype and allele distribution of *TNFRSF1A* and *IFNLR1* genes polymorphisms in individual cases (with breast cancer) and controls and logistic regression

				Model 1		Model 2	
Factor	Genotypes/ alleles	Controls: n (%)	Cases n (%)	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age		45.00 ± 16.14	53.75 ± 13.61	1.03 (1.02– 1.05)	**< 0.001**		
Smoking habit	Yes	32 (11.43%)	94 (40.69%)	5.32 (3.42– 8.46)	**< 0.001**		
	No	248 (88.57%)	137 (59.31%)	1			
BMI		25.79 ± 5.70	27.05 ± 5.29	1.04 (1.00– 1.08)	**0.018**		
TNFRSF1A rs1800693	CC	27 (10%)	11 (4.87%)	0.68 (0.29– 1.52)	0.357	0.74 (0.29– 1.83)	0.528
	CT	188 (69.63%)	182 (80.53%)	1.61 (1.00– 2.62)	**0.049**	1.60 (0.94– 2.74)	0.08
	TT	55 (20.37%)	33 (14.60%)	1		1	
	C	242 (44.81%)	204 (45.13%)	1.01 (0.79– 1.30)	0.92	1.03 (0.77– 1.37)	0.83
	T	298 (55.19%)	248 (54.87%)	1		1	
IFNLR1 rs4649203	AA	59 (21.53%)	32 (13.67%)	0.53 (0.28– 0.98)	**0.04**	0.38 (0.18 0.77)	**0.008**
	AG	178 (64.96%)	164 (70.09%)	0.89 (0.54– 1.48)	0.67	0.88 (0.50– 1.56)	0.67
	GG	37 (13.51%)	38 (16.24%)	1		1	
	A	296 (54.01%)	228 (48.72%)	0.80 (0.63– 1.03)	0.092	0.71 (0.54– 0.95)	**0.02**
	G	252 (45.99%)	240 (51.28%)	1		1	

Bold values indicate the statistical significance of $P < 0.05$

Model 1: univariate analysis; Model 2: multivariate analysis evaluating the effect of polymorphism corrected through age and smoking habit

n number; *OR* odds ratio; *CI* confidence interval; *BMI* body mass index

OR = 1.04, 95% CI: 1.00– 1.08) (Table 1). The smoking habit factor increases the risk of developing BC in around fivefold ($p < 0.001$, OR = 5.32, 95% CI: 3.42– 8.46) (Table 1). Thus, all tree features were associated with an increased risk for BC.

TNFRSF1A and IFNLR1 Gene Polymorphisms and the Risk of Breast Cancer

For *TNFRSF1A* gene the CT genotype was significantly associated with an increased susceptibility to BC when compared to the TT genotype ($p = 0.049$, OR = 1.61, 95% CI: 1.00–2.62, Table 1—Model 1). When adjusting to the significant factors (age, smoking habit, and BMI) obtained in the univariate model, the BMI factor was no longer significant. Was then chose to remove this factor from multivariate analysis, with only significant variables remaining in the final model. However, any

correlation was not found between *TNFRSF1A* polymorphism and breast cancer risk.

For *IFNLR1* gene, the AA genotype was significantly associated with reduced risk to BC when compared to the GG genotype ($p = 0.04$, OR = 0.53, 95% CI: 0.28–0.98 in Table 1). When performing the adjustment using the significant factors (age, smoking habit, and BMI) in the univariate model, the BMI factor again was no longer significant. Consistent with the results with *TNFRSF1A*, when removing BMI from multivariate analysis, the AA genotype remained significantly associated with a decreased risk for BC ($p = 0.008$, OR = 0.38, 95% CI: 0.28–1.09).

Considering the allele effects, no significant association with BC susceptibility in the univariate model was found, neither in the multivariate model for *TNFRSF1A* gene, even after adjustment. However, the *IFNLR1* A allele was a protective factor for BC ($p = 0.02$, OR = 0.71, 95% CI: 0.54– 0.95 in Table 1 – Model 2). Thus, the AA genotype and the A allele of the *IFNLR1* gene were related to a lower risk of developing breast cancer.

In addition to logistic regression, three genetic models (codominant, dominant, and recessive) were used to assess the association between genotypes and the susceptibility of BC (Table S1). For the *TNFRSF1A* variant, was found significant Chi-square value with the codominant model ($p = 0.014$) and recessive model ($p = 0.033$). For the *IFNLR1* variant, the recessive model showed a significant *p*-value of 0.021.

Associations Between the TNFRSF1A and IFNLR1 Polymorphisms and Clinical Features of the Patients

Among the patients under study, 58.97% were positive for ER, 47.86% for PGR, 11.96% for C-erbB-2, and 27.35% had affected lymph nodes. The associations between variants in the *TNFRSF1A* and *IFNLR1* genes and the clinical features of BC are shown in Table 2 and Tables S2–S4.

Only association was found between *IFNLR1* gene and the presence of PGR. The AA genotype was associated with the absence of progesterone receptor ($p = 0.02$, OR = 0.59, 95% CI: 0.38– 0.92 in Table 2). In the multivariate model adjusting for age, the AA genotype remained associated ($p = 0.02$, OR = 0.23, 95% CI: 0.06– 0.77 in Table 2). Thus, positive PGR is less prevalent in women with AA genotype than in those with GG genotype.

The A allele also showed significance ($p = 0.04$, PR = 0.86, 95% CI: 0.74–0.99 in Table 2) in the univariate model and multivariate analysis considering age ($p = 0.05$, PR = 0.63, 95% CI: 0.38–1.01 in Table 2).

Discussion

The current study investigated the associations between potentially functional variants in *TNFRSF1A* and *IFNLR1* genes with BC susceptibility and clinical and epidemiological data.

Contradictory data were found in the literature for the variant rs180693 T/C of the *TNFRSF1A* gene, regarding the association with other diseases. During previous

Table 2 Associations between *TNFRSF1A* and *IFNLR1* variants (genotypes and alleles) and progesterone receptor status in patients with breast cancer

		Model 1		Model 2	
Gene	Positive PRG	PR (95% CI)	*p*-value	PR (95% CI)	*p*-value
Age		0.99 (0.98—0.99)	**0.03**		
Smoking habit	Yes (n = 55)	1.15 (0.95—1.40)	0.15		
	No (n = 62)	1			
BMI		1.01 (0.99—1.03)	0.12		
TNFRSF1A rs1800693	CC (n = 8)	0.91 (0.49—1.69)	0.77	1.01 (0.17—6.54)	0.98
	CT (n = 126)	1.00 (0.73—1.40)	0.95	1.35 (0.43—3.86)	0.59
	TT (n = 19)	1		1	
	C (142)	0.99 (0.85—1.15)	0.91	1.02 (0.63—1.68)	0.92
	T (164)	1		1	
IFNLR1 rs4649203	AA (n = 25)	0.59 (0.38—0.92)	**0.02**	0.23 (0.06—0.77)	**0.02**
	AG (n = 110)	0.87 (0.70—1.08)	0.21	0.60 (0.19—1.64)	0.35
	GG (n = 27)	1		1	
	A (160)	0.86 (0.74—0.99)	**0.04**	0.63 (0.38—1.01)	**0.05**
	G (164)	1		1	

Model 1: univariate analysis; Model 2: multivariate analysis evaluating the effect of polymorphism corrected through age, smoking habit, and BMI

Abbreviations: n = number; PRG = progesterone receptor; PR = prevalence ratio; CI = confidence interval; BMI = body mass index. Bold values indicate the statistical significance of $P < 0.05$

results of a global population, meta-analyses indicated that T allele was associated with an increased risk of multiple sclerosis (MS) (De Jager et al. 2009). In a study with a UK cohort, on the other hand, the C allele was associated with MS, identifying increased expression of Δ6-TNFR1, a soluble form of the protein (Gregory et al. 2012). The C allele was also associated with an increased risk of MS in a case–control study conducted in a Germanic population (Hoffjan et al. 2015), in the population in the Slovak population (Javor et al. 2018), and in a recent study with the Kuwaiti population (Dashti et al. 2020). In addition, the C allele was associated with the risk to develop neuromyelitis optica in case–control study in a Korean population (Park et al. 2013). In a previous study with ankylosing spondylitis and disease severity assessed in a UK Caucasian population, CC homozygotes were found to have significantly worse Disease Activity Index than TT homozygotes (Watts et al. 2019). Noteworthy, to date only one study analyzed this variant in BC susceptibility, in a Chinese population, with the variant found associated only as part of a haplotype (Xu et al. 2014). In the present study, CT genotype was found associated with an increased risk of BC in the univariate model, but not in the multivariate model of logistic regression.

The functional consequence of rs1800693 variant of the *TNFRSF1A* gene and its role on disease development remains unclear. The variant is intronic and is located within a splice acceptor site and is believed to affect the splicing of the transcript. In an in vitro splicing assay, the G allele resulted in skipping of exon 6 leading to

the novel isoform Δ6-TNFR1, which lacks the extracellular C-terminal portion of the fourth cysteine-rich domain (CRD) of FL-TNFR1, the transmembrane domain, and the intracellular region which affects its appropriate subcellular localization (Gregory et al. 2012). This potentially affects its intracellular signaling, with possible effects on its biological function in the pathway. Thus, further studies would be important to better understand the pathways that the protein works and if the variant can affect the pathogenesis of diseases, such as BC, or if there is no association, as found in the present study.

In the present study, it was also shown that the rs4649203 variant in the *IFNLR1* gene is associated with BC and clinical data in a population from southern Brazil. The A allele and AA genotype were found to be protective factors for BC. This is the first report of the association of this gene with BC, only studies with autoimmune diseases have investigated this variant. In a case–control study, the A allele was associated with MS in the population of Bilbao (Lopez de Lapuente et al. 2012). A GWAS of several European countries found the A allele associated with psoriasis (Strange et al. 2010b). In contrast, G allele has been associated with psoriasis vulgaris and psoriasis arthritis in Chinese (Yang et al. 2013). A study in the Chinese Han population revealed that the G allele confers protection against psoriasis; in contrast, the same allele is associated with an elevated risk of systemic lupus erythematosus (Li et al. 2013). These results may suggest that the variant have opposing biological effects in these autoimmune diseases.

The functional effect of this polymorphism is not well known. However, it is located in the 3′UTR of the gene, suggesting that the variant may influence the expression of the IL28RA receptor (Gregory et al. 2012; Cai et al. 2015). The IL28RA interacts with INF-λ which has immunomodulatory properties, including the process of cellular apoptosis, important in the context of cancers (Numasaki et al. 2007; Li et al. 2009). The antiproliferative effects of IFN-λ have been demonstrated in various tumor cell lines that express IFN-λ receptors (Brand et al. 2005; Meager et al. 2005; Zitzmann et al. 2006). A genomic analysis of the IL28RA found that the molecule is expressed in different types of cancers, such as bladder cancer, BC, glioma, lymphoma, head and neck cancer, and lung cancer (Yang et al. 2010b). The same study identified that IL28RA expression was correlated with poorer BC patient survival (Yang et al. 2010b). The genetic association combined with further functional studies can contribute to the understanding of the mechanisms behind the association of IL28RA with BC and potentially other types of cancer.

The clinical features can in some cases predict BC prognosis and treatment outcomes. ER- and PGR-positive patients have a considerably better prognosis than ER- and PGR-negative when the patients are treated with hormone therapy. These two parameters have been commonly used as predictive markers for choice of treatment strategy (Stendahl et al. 2006; Rakha et al. 2019). The present study showed that the *IFNLR1* A allele was associated with PGR status suggesting that this variant may be important in predicting the prognosis of BC patients. This finding may indicate that this allele, when compared to G, may be related to a more stable mRNA, or an mRNA with less miRNAs binding sites and, consequently, might be related with higher expression of IL28RA receptor and thus enhanced anti-inflammatory activity, contributing to a better prognosis of BC patients.

Regarding epidemiological data, in this study smoking was related to a fivefold higher risk of developing BC. This result corroborates several other studies. Smoking may account for about 20–30% of all general cancer cases worldwide (Katzke et al. 2015). It is believed that the toxins contained in cigarettes can modulate the immune system, causing a reduction in the amount of NK cells and thus decreasing cell-mediated immunity (Scott et al. 2013). A study of nearly 80.000 women in the United States found that smokers were 16% more likely to develop BC than those who had never smoked (Luo et al. 2011). A meta-analysis with case–control studies stated that there is consistent evidence of a moderate increase in BC risk in women who smoke tobacco (Macacu et al. 2015). The relative risk of smoking-associated BC was also higher in women with a family history of the disease (Jones et al. 2017). It was also found that BMI factor was associated with increased risk for BC. Previous studies have also shown obesity as a risk factor (Ahn et al. 2007; Borgquist and Jirstr 2009; Chlebowski et al. 2013). A Brazilian study identified a 2.57-fold risk of developing BC in women with a BMI greater than 30 (Pinheiro et al. 2015). Another factor associated with the development of BC in this study was age. BC incidence and mortality increase proportionally with age. Throughout the world, this disease peaks around the age of 60 with a sharp incline beginning at age 40 (Winters et al. 2017).

The results found here together with further studies of the functional consequences of the variants might improve the understanding of the pathogenesis of BC and potentially other types of cancer. The association of genetic markers with particular clinical features of cancers is a huge step in the understanding of the different disease pathways and may represent important tools to the characterization, diagnostic, prognostic, and treatment of BC patients in more specific ways. These findings will be important in further understanding of the role of receptors *TNFRSF1A* and *IFNLR1* in BC development.

BC is a multifactorial chronic inflammatory disease involving a complex interplay between the person, environmental factors, and lifestyle habits. Like many other complex diseases, it is very difficult to investigate the influence of each factor involved in its pathogenesis individually, particularly the involvement of genetic factors. A few limitations of this study deserve to be mentioned. First, the small sample size. These observations should be confirmed in a larger sample of patients. Second, only one polymorphism in each gene was analyzed, and it would be worth considering other polymorphisms that are possibly functional in the *TNFRSF1A* and *IFNLR1* genes in future studies. However, the present study has some strengths, such as adjusting variables for many confounding variables, including age, habit smoking, and BMI. In addition, this is the first study to investigate the relationship between the *IFNLR1* gene variant with BC. This study has an adequate power to detect the effect of *IFNLR1* variant in breast cancer since the analyses conducted in this way were significant. However, this study was decided to analyze the clinical characteristics associated with the disease and observe several non-significant results. It was established that the power in the analyses shown in the supplementary tables is lower than the desirable value (80%). These low values can be one possible explanation to the not significant results observed since the sample size in these analyses were lower than that obtained in the susceptibility analysis. Besides that, for several

analyses the prevalence ratio observed was close to 1, showing that the groups were very similar. In these cases, the main reason for not significant results is a real no-difference between groups instead of the small sample size. However, it was the first study to analyze the effect of these variants in those characteristics. Further studies investigating other polymorphisms described for the two genes, as well as an investigation into how the polymorphisms can impact the expression of these proteins can help clarify the potential impact of these genetic variants on breast cancer pathways.

Conclusion

In conclusion, based on the outcomes obtained in the present analysis, it could be assumed that the AA genotype and the A allele for the rs4649203 variant of *IFNLR1* gene represent a protective factor for BC, and that the presence of the A allele or AA genotype is associated with the presence of progesterone receptor, possibly indicating a better prognosis. The study also found associations between the epidemiological data age, smoking habit and BMI and BC development. In this work no correlation was found between *TNFRSF1A* polymorphism and BC risk. Future studies should include investigation of these variants in larger populations and the analysis of gene expression in the context of breast cancer. These findings contribute to a better understanding of the involvement of these cytokine receptors in BC development and also in the search for markers that can be used to assist more specific diagnoses and prognoses, promoting a more individualized treatment.

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Compliance with Ethical Standards

Conflict of interest The authors declared no potential conflict of interests with respect to the research, authorship, and/or publication of this article.

Ethical Approval This work is part of a project approved by the Human Research Ethics Committee of the Universidade Federal de Santa Catatina (CEPSH-UFSC), protocol nº 922.167.

Consent to Participate Only the women who agreed to participate and gave their written informed consent were included in this study.

Consent for Publication We confirm that the manuscript has been read and approved by all named authors.

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