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Polymorphisms in cycloxygenase-2 gene and breast cancer prognosis: association between *PTGS2* haplotypes and histopathological features

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Abstract Cyclooxygenase-2 (COX-2) overexpression is associated with worse prognosis in breast cancer. COX-2 is encoded by a polymorphic gene, called *PTGS2,* and its expression may be genetically influenced. In this article, we investigate the association between *PTGS2* haplotypes and histopathological parameters with prognostic value on the clinical outcome of breast cancer. The study involved 606 women under current treatment for non-metastatic breast cancer. Patients were genotyped for rs689465, rs689466, rs20417, and rs5275, and their haplotypes were inferred. The distribution of *PTGS2* genotypes and haplotypes was evaluated according to histopathological categorical groups used for prognostic determination of low/intermediate versus high risk of tumor recurrence. Our results indicate positive associations between variant genotypes of rs689465 and estrogen receptor negativity (OR: 1.59, 95% CI: 1.04–2.44, *P*: 0.02) or HER2 positivity (OR: 1.79, 95% CI: 1.00–3.18, *P*: 0.03), and between variant genotypes of rs20417 and estrogen receptor negativity (OR: 1.75, 95% CI: 1.15–2.57, *P*: 0.005), progesterone receptor negativity (OR: 1.56, 95% CI: 1.09–2.22, *P*: 0.01) or HER2 positivity (OR: 1.80, 95% CI: 1.04–3.13, *P*: 0.02). In contrast, variant genotypes of rs689466 are negatively associated with estrogen receptor negativity (OR: 0.57, 95% CI: 0.33–0.98, *P*: 0.03). A total of eight haplotypes were inferred, and there was a significant difference in their distribution as a function of tumor size (*P*: 0.011), estrogen receptor status (*P*: 0.018), and HER2 status (*P*: 0.025). *PTGS2* haplotype *7 (formed by rs689465G, rs689466A, rs20417C, and rs5275T) is positively associated with higher tumor size (OR: 3.72, 95% CI: 1.19–11.22, *P*: 0.006), estrogen receptor negativity (OR: 2.43, 95% CI: 0.97–5.98, *P*: 0.032), progesterone receptor negativity (OR: 2.58, 95% CI: 1.05–6.39, *P*: 0.02), and HER2 positivity (OR: 4.17, 95% CI: 1.19–14.44, *P*: 0.007). Our results suggest that *PTGS2* haplotype *7 may contribute to higher growth of untreated breast cancer and that *PTGS2* haplotypes need to be considered in the characterization of breast cancer prognosis.

Keywords COX-2 · *PTGS2* · Polymorphisms · Haplotypes · Breast cancer · Histopathological features

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Introduction

Breast cancer is the most incident type of cancer (excluding non-melanoma skin cancer) and the commonest cause of cancer death among women worldwide. The annual incidence rates are higher in North America, Europe, and Australia, but are increasing in developing countries. The global annual incidence of breast cancer is estimated to be 1.4 million cases in 2008, and the expected mortality reaches 460,000 deaths [1].

The clinical course of the disease is highly variable and depends not only on the age and health status of the patient but also on the biology of the tumor [2]. The classical prognostic factors to forecast patient's survival are based

on morphological characteristics of the tumor, including size, histological type, and grade, as well as on the presence of metastasis in lymph nodes and in other sites. In addition to the morphological prognostic factors, the development of new systemic treatments based on specific targets has led to the characterization of molecular predictive factors of the tumor's responsiveness, such as the estrogen receptor and the epidermal growth factor receptor 2 (HER2).

The evaluation of individual prognostic and predictive factors is now incorporated to the clinical routine. However, the search for additional markers continues to further subcategorize patients, identifying those at higher risk of relapse, who would need intensive patient surveillance and who could benefit from additional or innovative therapies.

Cyclooxygenases are key enzymes in mediating the conversion of free arachidonic acid into prostaglandin H2, the precursor of molecules such as prostaglandins, prostacyclin, and thromboxanes [3]. Cyclooxygenase-2 (COX-2) overexpression is detected in several types of human cancers and in breast cancer, and it is associated with parameters of aggressiveness, including large tumor size, positive nodal status, angiogenesis, and lower survival [4, 5].

The mechanisms involved in the regulation of COX-2 expression remain unclear and may be influenced by genetic variations. COX-2 is encoded by a polymorphic gene, called *PTGS2*, with variant alleles located in the promoter region, next to binding sites for transcription factors [6, 7], and in the 3′-untranslated region, in regions involved with the maintenance of mRNA stability [8, 9].

A great number of studies have investigated the influence of *PTGS2* single nucleotide polymorphisms (SNPs) on the risk of developing different cancer types, including esophageal [10, 11], gastric [12, 13], prostate [14–18], colorectal [19–22], lung [23, 24], and breast cancer [25–36]. The results are conflicting, which may be accounted by the diversity of cancers and populations studied. Regarding breast cancer, the results of two meta-analyses indicate no strong risk association for *PTGS2* SNPs [35, 36], although this conclusion is restricted to SNPs rs20417 and rs5275. More recently, Brasky et al. [34] conducted a large case–control study involving various *PTGS2* SNPs, and their results confirm the lack of strong association between *PTGS2* haplotypes and breast cancer risk.

Although the results available until now do not suggest that *PTGS2* SNPs have great impact on the risk of developing breast cancer, it is not known whether they might have prognostic value on the disease progression. Only two papers evaluated the association between *PTGS2* SNPs and histopathological features in breast cancer, and they focused on isolated polymorphisms, not on haplotypes [27, 31].

Our group recently characterized the frequency of *PTGS2* SNPs among Brazilians [33], and identified four SNPs with frequencies higher than 0.10 (rs689465, rs689466, rs20417, and rs5275), which also appear to be the most frequent SNPs in other Western populations [19, 21, 23, 26, 28, 29, 34]. The aim of the present study was to investigate the association between *PTGS2* haplotypes formed by rs689465, rs689466, rs20417, and rs5275 and histopathological parameters with prognostic value on the clinical outcome of breast cancer.

Methods

Experimental design and study population

The study was conducted with women who were at least 18-years old and who were under current treatment for non-metastatic breast cancer at the Brazilian National Cancer Institute (INCA). The study was approved by the Ethics Committee of the Brazilian National Cancer Institute (Protocols #116/07 and #129/08), and all patients gave written consent to participate.

The study population involved two sets of patients. The first set was recruited between January and December 2008, and consisted of prevalent cases of breast cancer from a previous case–control study [33]. The second set was composed by recently diagnosed patients from a prospective cohort of incident cases, which was initiated in February 2009.

The exclusion criteria were: any previous oncological treatment, prior contralateral or bilateral synchronous breast cancer, systemic metastasis at the time of diagnosis, inability to answer the questions and patient's request to be excluded.

Clinical data collection

A total of 606 patients were included, and the histopathological characteristics of the tumors are presented in Table 1. The individual histopathological features were obtained from data available in electronic medical records. The histopathological analyses were performed after tumor resection and were based on TNM classification by the American Joint Committee on Cancer [37] and on the Elston Ellis histological grading system [38]. The following parameters were considered: Histological type (invasive or in situ); Tumor grade (G1—well differentiated, G2—moderately differentiated, and G3—poorly differentiated); Tumor size (based on the largest tumor dimension reported after surgical excision: T1 ($\leq$2 cm), T2 (2–5 cm), and T3 (>5 cm); Lymph node status: N0 (no metastasis in regional lymph nodes), N1 (metastasis in 1–3 lymph

Table 1 Histopathological features of the population

Prognostic factors	*N*	%
Histological Type		
Invasive	574	94.9
In situ	29	5.1
Missing data	3	
Ductal	560	94.4
Lobular	33	5.6
Missing data	13	
Tumor Grade (G)		
G1	63	12.4
G2	197	38.7
G3	249	48.9
Missing data	97	
Tumor size (T)		
Tis	29	5.3
T1	205	37.4
T2	249	45.4
T3	65	11.9
Missing data	58	
Nodal status (N)		
N0	304	54.6
N1	130	23.3
N2	79	14.2
N3	44	7.9
Missing data	49	
ER status		
Positive	445	76.0
Negative	140	24.0
Missing data	21	
PR status		
Positive	365	63.0
Negative	214	37.0
Missing data	27	
HER2 status		
Positive	71	18.7
Negative	309	81.3
Missing data	226	

The percentages were calculated according to the valid data

Tis carcinoma in situ; *ER* estrogen receptor; *PR* progesterone receptor; *HER2* epidermal growth factor receptor 2

nodes), N2 (metastasis in 4–9 lymph nodes), and N3 (metastasis in 10 or more lymph nodes); Hormone receptors (estrogen and progesterone) and HER2 status.

Genotyping

The patients from the case–control study had already been genotyped for *PTGS2* SNPs rs689465, rs689466, rs20417, and rs5275 [33], and those with positive identification for the four SNPs ($N = 231$) were selected for the present study.

The patients recruited in the prospective cohort ($N = 375$) were genotyped for SNPs rs689465 and rs20417 using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) as described previously [33], whereas the SNPs rs689466 and rs5275 were identified by allelic discrimination using TaqMan® Probe (Applied Biosystems, Warrington, UK). Primers and FAM- and VIC-labeled probes were purchased from Applied Biosystems (rs689466 probe: C___2517145_20; rs5275 probe: C___7550203_10). All assays were carried out in 96-well plates. Each plate included negative controls (with no DNA) and positive controls, which were previously analyzed by automatic sequencing using ABI PRISM-377 equipment (TaqMan, PE Biosystems, Foster City, CA, USA). Plates were read on the ABI Prism 7500 (Applied Biosystems, Warrington, UK) using the Sequence Detection Software (7500 Fast System SDS Software v1.4). One set of randomly selected samples was used as blind duplicated samples to check the reproducibility of the genotyping assays, and the results matched completely. There were no failed genotypes in this new set of patients.

Statistical analysis

Allelic and genotypic frequencies were derived by gene counting, and the adherence to the Hardy–Weinberg principle was evaluated by the Chi-square test for goodness of-fit.

The haplotype patterns were inferred using Haploview 4.2 (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview), based on the algorithm of expectation and maximization [39]. Comparisons of haplotypic distribution between histopathological categorical groups were performed using the Chi-square test for proportions.

The association between selected histopathological features and identified genotypes or inferred haplotypes was estimated by the odds ratio (OR) with 95% confidence interval (95% CI). The threshold for significance was set at $P < 0.05$. All statistical analyses were conducted using SPSS 13.0 for Windows (SPSS Inc, Chicago, Illinois).

Results

Association between *PTGS2* genotypes and histopathological features in breast cancer

The minor allele frequencies for the four *PTGS2* SNPs were: rs689465G (0.16), rs689466A (0.12), rs20417C

(0.27), and rs5275T (0.36). All genotypic distributions followed Hardy–Weinberg equilibrium.

The distribution of *PTGS2* genotypes was evaluated according to histopathological categorical features, and significant associations were found only for receptor status (Table 2). Our results indicate positive associations between variant genotypes of rs689465 (AG + GG) and estrogen receptor negativity (OR: 1.59, 95% CI: 1.04–2.44, *P*: 0.020) or HER2 positivity (OR: 1.79, 95% CI: 1.00–3.18, *P*: 0.034), and between variant genotypes of rs20417 (GC + CC) and estrogen receptor negativity (OR: 1.75, 95% CI: 1.15–2.57, *P*: 0.005), progesterone receptor negativity (OR: 1.56, 95% CI: 1.09–2.22, *P*: 0.010) or HER2 positivity (OR: 1.80, 95% CI: 1.04–3.13, *P*: 0.025). In contrast, variant genotypes of rs689466 (AG + GG) are negatively associated with estrogen receptor negativity (OR: 0.57, 95% CI: 0.33–0.98, *P*: 0.030). No significant differences in receptor status were found in relation to rs5275, either considering combined variant genotypes (Table 2) or comparing variant homozygotes in relation to the other two genotypes (data not shown).

Association between *PTGS2* haplotypes and histopathological features in breast cancer

Table 3 shows the distribution of *PTGS2* haplotypes among the patients. A total of eight haplotypes could be inferred, which account for more than 99% of the study population. The distribution of *PTGS2* haplotypes according to histopathological categories used for prognostic determination of patients at low/intermediate risk versus high risk of recurrence is shown in Table 4. The results indicate a significant difference in the distribution of *PTGS2* haplotypes in relation to tumor size (*P*: 0.011), estrogen receptor status (*P*: 0.018), and HER2 status (*P*: 0.025). In order to investigate specific associations, the distribution of each variant haplotype in relation to each histopathological categorical feature was compared with haplotype *1. The results indicate significant associations only for haplotype *7 (Table 5), which is positively associated with higher tumor size (OR: 3.72, 95% CI: 1.19–11.22, *P*: 0.006), estrogen receptor negativity (OR: 2.43, 95% CI: 0.97–5.98, *P*: 0.032), progesterone receptor negativity (OR: 2.58, 95% CI: 1.05–6.39, *P*: 0.02), and HER2 positivity (OR: 4.17, 95% CI: 1.19–14.44, *P*: 0.007).

Discussion

A great number of studies have investigated the influence of *PTGS2* SNPs on the risk of developing different cancer types, but very few focused on their role on the progression of the disease. In addition, most studies evaluated only one

Table 2 *PTGS2* genotypes and receptor status

SNP	Genotype	Receptor status													
		ER+	ER−	PX^2	OR	95% CI	PR+	PR−	PX^2	OR	95% CI	HER2−	HER2+	PX^2	OR	95% CI
rs689465	AA	330	90	**0.020**	**1.59**	**1.04–2.44**	269	147	0.195	1.28	0.87–1.88	230	44	**0.034**	**1.79**	**1.00–3.18**
	AG/GG	115	50				96	67				79	27			
rs689466	AA	340	119	**0.030**	**0.57**	**0.33–0.98**	279	175	0.131	0.72	0.46–1.13	240	61	0.122	0.57	0.26–1.22
	AG/GG	105	21				86	39				69	10			
rs20417	GG	257	62	**0.005**	**1.72**	**1.15–2.57**	214	102	**0.010**	**1.56**	**1.09–2.22**	180	31	**0.025**	**1.80**	**1.04–3.13**
	GC/CC	188	78				151	112				129	40			
rs5275	TT	182	54	0.624	1.1	0.73–1.66	154	81	0.304	1.20	0.84–1.72	130	27	0.532	1.18	0.68–2.08
	TC/CC	263	6				211	133				179	44			

P values are presented for the comparison of genotypic distribution between the two categories of each histopathological parameter

ER estrogen receptor; *PR* progesterone receptor; *HER2* human EGF receptor 2

Boldness was used to highlight statistically significant ($P < 0.05$) associations

Table 3 Haplotype characterization

Haplotype	SNP				Frequency
	rs689465	rs689466	rs20417	rs5275	
*1	A	A	G	T	0.463
*2	A	A	G	C	0.133
*3	G	A	C	C	0.113
*4	A	G	G	T	0.111
*5	A	A	C	C	0.092
*6	A	A	C	T	0.041
*7	G	A	C	T	0.022
*8	G	A	G	C	0.019

The frequencies of *PTGS2* haplotypes were inferred using the computer software Haploview4.2

or a few SNPs at a time, sometimes with no clear selection criteria. In a previous study, we screened 1.5 kb of the PR and 1.2 kb of the 3′-UTR to identify the most frequent SNPs in the regulatory sites of *PTGS2* [33]. The focus on the regulatory regions of the gene is justified by the fact that *PTGS2* mRNA is very unstable, and an increase in gene transcription [7] or in mRNA stability [40] would promote COX-2 expression. The SNPs rs689465, rs689466, rs20417, and rs5275 were identified as the most frequent SNPs in Brazilians, and also appear to be the most frequent *PTGS2* SNPs in other Western populations [19, 21, 23, 26, 28, 29, 34].

In vitro studies indicate possible functional effects of the SNPs rs689466 and rs20417 on *PTGS2* promoter activity (evaluated by a luciferase gene reporter system) [6, 7] and on COX-2 activity (evaluated by PGE2 production in human monocytes) [41]. A recent report by Moore et al. [42] indicates that rs5275 disrupts micro-RNA-mediated regulation of COX-2 mRNA degradation, which leads to an increase in mRNA stability and in COX-2 protein levels. The four *PTGS2* SNPs form various haplotypes which differ in frequency among different populations [11, 33, 43], and which may have diverse influences on *PTGS2* expression and COX-2 activity. In agreement with the latter notion, Sanak et al. [44] showed that instead of genotypes, haplotypes of *PTGS2* could better correlate with prostaglandins biosynthetic capacity. They observed very strong linear correlation between the number of rs20417C–rs5275C haplotype copies and the levels of prostaglandins in monocyte cultures [C–C/C–C diplotypes revealed a 19.8-fold increase of PGE2 ($P < 0.001$) in relation to the wild diplotype (G–T/G–T)].

As far as we know, the present work is the first study to focus on the possible contribution of the four most frequent *PTGS2* SNPs and its haplotypes on the progression of breast cancer. We investigated the association between the four *PTGS2* SNPs and histopathological features used

Table 4 *PTGS2* haplotype distribution based on histopathological categorical features

Haplotype					Tumor size		Grade		Nodal status		Receptor status					
					T1 and T2 ($N = 908$)	T3 ($N = 130$)	G1 and G2 ($N = 520$)	G3 ($N = 498$)	N0 ($N = 608$)	$N \geq 1$ ($N = 506$)	ER+ ($N = 890$)	ER− ($N = 280$)	PR+ ($N = 730$)	PR− ($N = 428$)	HER2− ($N = 618$)	HER2+ ($N = 142$)
***1**	A	A	G	T	0.476	0.446	0.465	0.478	0.483	0.450	0.473	0.444	0.480	0.444	0.481	0.428
***2**	A	A	G	C	0.128	0.103	0.139	0.122	0.130	0.130	0.135	0.109	0.134	0.116	0.125	0.111
***3**	G	A	C	C	0.111	0.151	0.097	0.122	0.129	0.100	0.105	0.144	0.108	0.125	0.112	0.135
***4**	A	G	G	T	0.111	0.115	0.111	0.110	0.097	0.128	0.124	0.073	0.121	0.097	0.123	0.077
***5**	A	A	C	C	0.104	0.043	0.095	0.100	0.086	0.104	0.087	0.114	0.080	0.120	0.089	0.112
***6**	A	A	C	T	0.033	0.054	0.038	0.036	0.038	0.038	0.034	0.051	0.034	0.044	0.031	0.068
***7**	G	A	C	T	0.013	0.047	0.033	0.011	0.013	0.025	0.016	0.035	0.014	0.033	0.012	0.044
***8**	G	A	G	C	0.020		0.017	0.017	0.021	0.017	0.019	0.019	0.021	0.018	0.026	0.015
P value					**0.011**		0.298		0.293		**0.018**		0.065		**0.025**	

P values are presented for the comparison of haplotypic distribution between the two categories of each histopathological parameter

ER estrogen receptor; *PR* progesterone receptor; *HER2* human EGF receptor 2; *N* corresponds to the number of chromosomes

Boldness was used to highlight statistically significant ($P < 0.05$) associations

Table 5 *PTGS2* haplotype *7 and histopathological categorical features

Histopathological categories	Haplotype *1 *N*	Haplotype *7 *N*	OR	95% CI	PX^2
Tumor Size					
T1 and T2	432	12			
T3	58	6	3.72	1.19–11.22	**0.006**
ER					
ER+	421	14			
ER−	124	10	2.43	0.97–5.98	**0.032**
PR					
PR+	350	10			
PR−	190	14	2.58	1.05–6.39	**0.020**
HER2					
HER2−	297	7			
HER2+	61	6	4.17	1.19–14.44	**0.007**

P values are presented for the comparison of categorical distribution between the two haplotypes

ER estrogen receptor; *PR* progesterone receptor; *HER2* human EGF receptor 2; *OR* odds ratio; *CI* confidence interval; *N* number of chromosomes

Boldness was used to highlight statistically significant ($P < 0.05$) associations

for prognostic estimation of the risk of recurrence. The first report on the association between *PTGS2* SNPs and histopathological features of breast cancer was performed by Langsenlehner et al. [27], who evaluated the distribution of rs5275 in 500 patients from Austria. They found that estrogen receptor positivity was less frequent among carriers of the CC genotype (63.9%) than among carriers of a TT or TC genotype (76.9%; *P*: 0.028). In our population, the estrogen receptor positivity was 68% among carriers of the CC genotype and 77% among carriers of a TT or TC genotype, but no significant association was found (OR: 1.54, 95% CI: 0.86–2.74; *P*: 0.11, data not shown).

There are no previous literature reports regarding the association between the other *PTGS2* SNPs (rs689465, rs689466, or rs20417) and prognostic histopathological features of breast cancer. Our results indicate that the variant genotypes of both rs689465 and rs20417 are significantly associated with estrogen receptor negativity and with HER2 positivity, and that rs20417 is also associated with progesterone receptor negativity. These characteristics are indicative of a worse prognosis for rs689465 and rs20417, since estrogen receptor and progesterone receptor are associated with improved outcomes and are predictive of response to endocrine therapy [45], whereas overexpression of HER2 is correlated with decreased relapse-free and overall survival, and resistance to hormonal and cytotoxic therapy [46–48]. In contrast, variant genotypes of rs689466 were associated with estrogen receptor positivity, suggesting a better prognosis for individuals with this SNP.

The interpretation of the single-SNP analysis could lead to the conclusion that rs5275 is irrelevant, since no independent associations were found between rs5275 and any breast cancer histopathological features. Nevertheless, if the distribution of haplotypes is inferred without rs5275, the significant difference in relation to tumor size is lost, whereas the significant differences in relation to hormonal receptors and HER2 status are maintained (data not shown). This result suggests that the association between haplotype *7 and tumor size depends on rs5275, whereas the associations with hormonal receptors and with HER2 status are mostly dependent on the other three SNPs. This result outlines the importance of haplotype analysis for the study of *PTGS2* SNPs in cancer.

The association between haplotype *7 and higher tumor size at diagnosis, and the lack of such association in relation to haplotype *3, which differs from haplotype *7 only by the presence of rs5275C, may imply a protective role for rs5275C in relation to the growth of untreated breast cancer. This supposed protective role of rs5275C, however, would not be presumed according to the results by Moore et al. [42] or by Sanak et al. [44], and was not detected when the genotypes were analyzed independently. Gerger et al. [49] and Abraham et al. [30] evaluated the role of rs5275 on the survival of breast cancer patients. The authors found no significant associations between rs5275 and disease-free [49] or overall survival [30], but they did not consider *PTGS2* haplotypes in their analyses.

In a previous study, we showed that SNPs rs689465, rs20417, and rs5275 present significant pairwise linkage disequilibrium, with their minor alleles often occurring simultaneously, whereas the rs689466 G allele occurs mostly as an isolated variation [33]. The haplotype analysis in this study indicates that rs689466 could be used as a tagSNP of haplotype *4 and this approach may be

considered in the future. The other SNPs, however, are not fully informative of *PTGS2* haplotypes, and at least SNPs rs689465 and rs5275 are required to infer haplotype *7, whereas the four *PTGS2* SNPs are necessary for characterization of the eight most frequent *PTGS2* haplotypes.

Li et al. [31] recently described a new *PTGS2* SNP, located in exon 2, called "*169CG*" (no rs available). The authors evaluated 310 breast cancer patients from China and found that estrogen receptor positivity was less frequent among carriers of the GG genotype (61.7%) than among carriers of CC or GC genotypes (72.3%; *P*: 0.02). There is no description of the SNP *169CG* in Western populations. However, it would be necessary to perform genotyping assays to characterize the frequency of this SNP among Brazilians and to evaluate if it alters the distribution of *PTGS2* haplotypes.

The main limitation of this study is that the prognostic evaluation of *PTGS2* SNPs and its haplotypes in breast cancer is only associative, with regards to well-established histopathological features used for estimation of the recurrence risk. The best approach to investigate the independent effects of *PTGS2* SNPs on the progression of the disease would be a survival analysis in a prospective cohort of incident cases. Although we are currently recruiting with this purpose, our cohort has only 2 years of follow-up, which is a very short time for survival analyses in breast cancer. Another limitation is that the tumors were only classified according to the histopathological features, since gene expression profiling analyses were not available to characterize molecular subtypes, such as luminal A and B, basal or HER2 enriched, which could possibly enable better prognostic evaluation [50–52]. Finally, the confidence of the results regarding haplotype *7 is compromised by the low frequency of this haplotype and by the relatively small size of the population studied until now.

In conclusion, our findings suggest that haplotype *7 of *PTGS2* may contribute to higher growth of untreated breast tumors, but more importantly, they point to the need of considering haplotypes, instead of genotypes, when evaluating the role of *PTGS2* SNPs in the physiopathology of breast cancer.

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Conflict of interest The authors declare that they have no conflicts of interest.

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