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Keywords: *Breast cancer, CYP17, MTRR, polymorphism.*

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Association of *CYP17* and *MTRR* Gene Polymorphisms with Clinicopathological Features of Breast Cancer Patients

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Abstract- Allele frequencies of T-34C *CYP17* and A66G *MTRR* polymorphisms in breast cancer samples and the correlation with clinicopathological data can contribute to the prognosis and knowledge of the genetic profile of a population. In this study, was analized the association of T-34C *CYP17* and A66G *MTRR* polymorphisms with clinicopathological data in 82 samples of invasive ductal breast carcinoma in the Southwest region of Bahia. PCR-RFLP was used to determine the genotypes for A66G *MTRR* and T-34C *CYP17* polymorphisms. The allele frequency was 0.369 and 0.631 for A66G *MTRR*, 0.672 and 0.328 for T-34C *CYP17*. The A66G *MTRR* genotypes showed deviation from Hardy–Weinberg equilibrium (p=0.000), the genotypes are not segregating independently (p=0.036). No association of polymorphisms with clinicopathological features was observed.

Keywords: Breast cancer, CYP17, MTRR, polymorphism.

I. Introduction

According to the International Agency for Research on Cancer (IARC, 2019), breast cancer is the most prevalent neoplasm among women worldwide, with invasive ductal carcinoma (IDC) of the breast being the most common histological type, corresponding to about 80%. Like all cancers, breast cancer is a multifactorial disease with environmental and genetic factors as causes (Rojas & Stuckey, 2016).

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It is used several clinical and pathological factors to define the prognosis of the disease as well as to determine the most appropriate therapy. These factors include demographic (age, preand postmenopausal status and ethnicity) and the tumor characteristics (affected axillary lymph nodes, tumor size, type and histological grade, expression of hormone receptors, and HER2) (Schnitt, 2010). Also, studies of genetic polymorphisms associated with breast cancer has contributed to the understanding of the biology of this disease as well as to the discovery of new genetic susceptibility markers that may assist in the prognosis and therapeutic management of the disease (Lilyquist, Ruddy, Vachon & Couch, 2018; Low, Zembutsu, & Nakamura, 2018).

Polymorphisms of the *CYP17* and *MTRR* genes have been the target of studies since they are related to pathways for breast carcinogenesis: estrogen biosynthesis and methionine biosynthesis (Mo, Ding, Zheng, Zou & Ding, 2020; Sun et al., 2018). *MTRR* gene codes for the enzyme methionine synthase reductase which is responsible for the active state of the enzyme MTR (methionine synthase), which catalyzes the addition of a methyl group to homocysteine thus forming methionine. SAM (S-adenosylmethionine) receives the methyl group of methionine, the universal donor molecule of the methyl group responsible for the methylation profile of DNA (Bottiglieri, 2005; Hiraoka & Kagawa, 2017; Weiner et al., 2012). Studies of the A66G polymorphism of the *MTRR* gene indicate that the G allele decreases the activity of the MTRR enzyme, thus being able to influence homocysteine levels (Olteanu, Munson & Banerjee, 2002). Therefore, disturbances in this metabolic pathway are associated with the carcinogenesis process as they interfere in the pathways responsible for maintaining the pattern of DNA methylation of the cell (Hasan et al., 2019).

The *CYP17* gene codes for a cytochrome P450 enzyme. This enzyme participates in two stages of estrogen biosynthesis from cholesterol (Guo et al., 2006). One of the polymorphisms of the *CYP17* gene is the T-34C located in the 5´ UTR (5´untraslated region) of the promoter. This mutation potentiates promoter

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activity by increasing *CYP17* expression (Carey et al., 1994) and estrogen levels (Clemons & Goss, 2001), which is associated with an increased risk of breast cancer (Wen, Wu, Fu, Wang, & Zhou, 2017).

The frequency of polymorphic alleles observed in the population can show an ethnographic variation (Binia et al., 2014). The Brazilian, and especially the population of the state of Bahia, is known to be highly admixture because of the initial composition formed by Amerindians, European, and African descendants (Abé-Sandes, Silva Junior & Zago, 2004). The knowledge of the frequencies of the polymorphic alleles of *CYP17* and *MTRR* in the samples of invasive ductal breast carcinoma and the correlation of these alleles with clinical and pathological characteristics can contribute to the knowledge of the prognostic and genetic profile of women the Northeast of Brazil

Thus, this study analyzed the combined association of T-34C *CYP17* and A66G *MTRR* polymorphisms with clinical and pathological aspects (age, tumor size, histological grade, and lymph node involvement) in patients with invasive ductal breast carcinoma in the Southwest region of Bahia.

II. Methods

a) Subjects

Approval was obtained by the Research Ethics Committee of the State University of Southwest Bahia (UESB) Vitoria da Conquista, Brazil. The population of interest was composed of 82 unrelated subjects with histopathological diagnosis of invasive ductal breast carcinoma.

b) Genotype determination

The DNA was extracted from tumoral breast tissue embedded in a paraffin block using the QIAamp DNA FFPE Tissue (https://www.qiagen.com/us/). Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism (PCR-RFLP) was used to determine genotypes for the two polymorphic regions A66G *MTRR* and T-34C CYP17 using the primer strings: (F) 5'GCAAAGGCCATCGCAGAAGACAT3' and (R) 5'GTGAAGATCTGCAGAAAATCCATGTA3' (Wilson et al., 1999) and (F) 5′CAAGGTGAAGATCAGGGTAG3′ and (R) 5′GCTAGGGTAAGCAGCAAGAG3′ (Kuligina et al., 2000), respectively. Was performed a PCR according to the following protocol: 2,5 μM reaction buffer 10x (Invitrogen), 2,5 mM $MgCl_2$ (Invitrogen), 1,25 mM dNTPs (Invitrogen), 2,5 mM of each primer (Invitrogen), 1U of Taq DNA polymerase (Invitrogen). Sample were exposed to 94°C for 5 min (initiation), 35 cycles at 94°C for 30s (denaturation), 60°C (A66G *MTRR*) or 57°C (T-34C *CYP17*) for 40s (annealing) and 72°C for 30s (extension). The reaction was finalized with the extension at 72°C for 5 minutes. The check of the PCR products was on a 3% agarose gel stained with ethidium bromide and visualized an L-PIX HE transilluminator (Locus Biotechnology). For A66G MTRR and T-34C CYP17 were observed fragments of 66 bp (base pairs) and 145 bp, respectively.

The digest of the PCR product A66G *MTRR* (66 bp) was performed by the *NdeI* restriction enzyme (Thermo Scientific) at 37°C for 1 hour (Wilson et al., 1999). The substitution A>G eliminates the restriction site for the *NedI* enzyme. Therefore, after digestion, wild homozygotes (AA) generate fragments of 44 bp and 22 bp, and mutant homozygotes (GG) were not digested, remaining at 66 bp. Heterozygotes (AG) have fragments of 66, 44, and 22 bp after digestion. The digestion product was checked on 10% polyacrylamide gel and subsequently visualized after staining with silver nitrate.

The digest of the PCR product of polymorphism T-34C *CYP17* (145 bp) was used the *MspA1* restriction enzyme (Thermo Scientific) at 37°C for 4 hours (Kuligina et al., 2000). The substitution T>C generate a restriction site for the *MspA1* enzyme. Were generated fragments of 145 bp; 75 and 70 bp; and 45, 75 and 70 bp after digestion for wild homozygous (TT), mutant homozygous (CC), and mutant heterozygous (TC), respectively. The check of the digest products was on a 5% agarose gel stained with ethidium bromide.

c) Statistical Analysis

Analyses of the Hardy–Weinberg equilibrium and Linkage disequilibrium for unconnected loci were made for each polymorphism, both using Genepop (4.2 version). The $\chi 2$ tests were used for analyses of differences in genotype frequency. The association between the genetic polymorphisms A66G *MTRR* and T-34C *CYP17* and clinical-pathological features were determined by odds ratio (OR) and corresponding 95% confidence intervals (95% CIs). We compared A66G *MTRR* and T-34C *CYP17* alleles and genotype distributions in subgroups of subjects (age: >49 and <49; histological grade: I+II and III+IV; tumor size: <3 and >3; lymph node involvement: yes and no).

III. Results

Were included eighty-two women in this study. Clinical-pathological features were available *(Table 1)*. Fifteen (18.3%) subjects are under 49 years of age at the time of diagnosis. Most have histological grade II (42.7%) and tumor size >3 cm (71.9%). Lymph node involvement was present in (54.9%) of subjects (Table 1).

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Table 1: Clinical-pathological features of the 82 unrelated breast cancer subjects

Clinical-pathological features	Number (%)
Age (years)	
30-49	15 (18.3%)
50-69	32 (39%)
70-99	32(39%)
Unknown	3 (3.7%)
Histological grade	
I	15 (18.3%)
II	35 (42.7%)
III	17 (20.7%)
IV	1 (1.2%)
Unknown	14 (17%)
Tumor size (cm)	
<3	23 (28.1%)
>3	59 (71.9%)
Lymph node involvement	
Yes	45 (54.9%)
No	23 (28.1%)
Unknown	14 (17.1%)

The allele frequency was 0.369 and 0.631 for A66G *MTRR* polymorphism; 0.672 and 0.328 for T-34C *CYP17* polymorphism. The distribution of genotypes of T-34C *CYP17* polymorphism showed no deviation from Hardy–Weinberg equilibrium ($p=0.278$). However, A66G *MTRR* polymorphism not aligned to Hardy–Weinberg equilibrium ($p=0.000$), were found at higher and low frequency for the AG and AA genotypes, respectively *(Table 2)*. Analyses of genotypic linkage disequilibrium showed that the genotypes were not segregating independently ($p=0.036$). No allele or genotype for A66G *MTRR* and T-34C *CYP17* were associated with the clinical-pathological features of subjects *(Table 3)*.

Table 2: Allele and genotype frequencies of polymorphic regions A66G *MTRR* and T-34C *CYP17* in 82 unrelated breast cancer subjects.

Genotype or allele	Frequency (%)	$\chi 2$	*p-value*
A66G MTRR			
A	0.369		
G	0.631		
AA	2.5	8.10	**0.01**
AG	68,8	8.75	**0.01**
GG	28,7	2.53	0.2
T-34C CYP17			
T	0.672		
C	0.328		
TT	41.8	0.13	0.95
TC	50.7	0.53	0.70
CC	7.5	0.57	0.70

Abbreviations: $\chi 2$: chi-square. Statistically significant: $p=0.05$.

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Table 3: Odds ratio of clinical-pathological features between polymorphic regions A66G MTRR and T-34C CYP17 in 82 unrelated breast cancer subjects.

Genotypes or alleles	Clinical-pathological features							
	Age (>49 and <49)		Histological grade (I+II and III+IV)		Tumor size (<3 and >3)		Lymph node involvement (yes and no)	
A66G MTRR	**OR (CI 95%)**	***p-value***	**OR (CI 95%)**	***p-value***	**OR (CI 95%)**	***p-value***	**OR (CI 95%)**	***p-value***
A	1.00 (Reference)	-	1.00 (Reference)	-	1.00(Reference)	-	1.00(Reference)	-
G	1.12 (0.52-2.43)	0.76	0.93 (0.45-1.94)	0.85	0.91 (0.49-1.75)	0.79	0.99 (0.50-1.93)	0.97
AA	1.00 (Reference))	-	1.00 (Reference)	-	1.00 (Reference)	-	1.00 (Reference)	-
AG	0.95 (0.39-2.26)	0.90	1.02 (0.44-2.35)	0.96	0.96 (0.45-2.05)	0.91	1.02 (0.46-2.27)	0.96
GG	1.45 (0.38-5.58)	0.59	0.82 (0.23-2.85)	0.75	0.86 (0.31-2.39)	0.78	0.94 (0.33-2.70)	0.92
T-34C CYP17								
T	1.00 (Reference)	-	1.00 (Reference)	-	1.00 (Reference)	-	1.00 (Reference)	-
C	0.75 (0.30-1.86)	0.53	0.46 (0.14-1.56)	0.21	0.91 (0.40-2.07)	0.82	1.45 (0.55-3.84)	0.45
TT	1.00 (Reference)	-	1.00 (Reference)	-	1.00 (Reference)	-	1.00 (Reference)	-
TC	1.00 (0.35-2.89)	0.99	0.67 (0.19-2.23)	0.49	0.61 (0.26-1.44)	0.26	1.40 (0.48-4.11)	0.54
CC	0.36 (0.05-2.36)	0.29	0.45 (0.02-9.26)	0.60	4.75 (0.2589.89)	0.29	1.60 (0.16-15.44)	0.68

Abbreviations: odds ratio (OR); confidence intervals (CI).Statistically significant: p=0.05

IV. Discussion

Over the past few years, studies on the association between the A66G *MTRR* and T-34C *CYP17* polymorphisms with breast cancer have been controversial, which has confirmed in the meta-analyses carried out for both the A66G *MTRR* polymorphism (Hu, Zhou, Wang, & Wang, 2010; Mo et al., 2020; Wang, Li, Wang, He, & Xi, 2017) and for the T-34C *CYP17* (Chen & Pei, 2010; Sun et al., 2018) with a greater tendency towards the absence of association. In this sense, should be considering that the frequency of the analyzed alleles can vary according to the population studied (Binia et al., 2014; Kato, Cichon, Yee, Land, & Korczak, 2009) and that the cancer is a multifactorial disease. Not only genetic factors (genes with high, low and moderate penetrance)(Apostolou & Fostira, 2013; Shiovitz & Korde, 2015) but also environmental factors contribute together to the risk of developing breast cancer (Apostolou & Fostira, 2013; Stratton & Rahman, 2008; Syamala et al., 2010).

In this study, conducted with 82 women with breast IDC in the southwestern region of Bahia, the analyzes performed did not indicate an association between the A66G *MTRR,* T-34C *CYP17* polymorphisms with clinical-pathological aspects such as age, tumor size, and histological grade. The analyzes showed an excess of heterozygotes for the *MTRR* locus, indicating a deviation from the Hardy-Weinberg principle. Additionally, the genotypes are not segregating independently. These findings may be due the probable admixture of the studied population, as well as the effect of the distribution of genotypic frequencies in samples of women with breast IDC not being random.

In a population in Canada was not found an association between the *CYP17* polymorphism and the increased risk for breast cancer and the degree of the tumor. However, their results suggest that the gene polymorphisms that control the formation and availability of estrogen interact significantly with other risk factors such as estrogen receptor (ER) status, use of oral contraceptives and pre-menopause, influencing an increased risk for this neoplasm (Cribb et al.,2011). In a study conducted with Chinese women, it was found that the presence of the TC genotype significantly increased the risk of postmenopausal breast cancer (Zhang et al., 2009). Also, other evidence indicated a possible impact on menopausal status, age at menarche, and BMI (Body Mass Index) in the association between the *CYP17* T-34C polymorphism and the risk of breast cancer, as verified by a meta-analysis (Chen & Pei, 2010).

Regarding the *MTRR* polymorphism, although studies indicate that this polymorphism does not confer an increased risk for breast cancer (Hu et al., 2010; Weiner et al., 2012), work carried by Suzuki et al., (2008) pointed that polymorphisms *MTRR* and *MTHFR* were associated with individual susceptibility to breast cancer in post-menopausal women. The reported studies, therefore, demonstrate a probable association of these polymorphisms with other clinical factors not evaluated by us, such as menopausal status, age at menarche, and BMI, aspects that are not available for our analyzes.

Studies of the association of genetic polymorphisms with clinical and pathological aspects in different neoplasms seek to contribute to the knowledge of the prognostic profile of patients and thus collaborate not only in the diagnosis and establishment of the best treatment but also in the prevention of the disease. However, the frequencies of alleles can differ depending on the population studied, and it is important that these types of studies are carried out in different populations to establish the genetic profile of each region.

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The limitation of this study is the low number of samples and the absence of controls. Thus, the expansion of the sample number, as well as the analysis of the frequencies of these polymorphisms in control samples, may provide a better understanding of the effect of these polymorphisms on breast cancer in our population.

V. Conclusions

Altogether, the data did not indicate an association between the A66G of *MTRR* and T-34C of *CYP17* polymorphisms with some clinicopathological features of invasive ductal breast carcinoma. Although these findings need further validation, our data contribute to the analysis of the genetic profile of women with breast cancer in the Northeast of Brazil and understanding diverse aspects of breast cancer biology.

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