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

Sandra Mara Bispo Sousa

Received: 16 December 2019 Accepted: 5 January 2020 Published: 15 January 2020

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 Hardyâ??"Weinberg equilibrium (p=0.000), the genotypes are not segregating independently (p=0.036). No association of polymorphisms with clinicopathological features was observed.

Index terms— Breast cancer, CYP17, MTRR, polymorphism.

1 Introduction

ccording 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).

2 A

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 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.

3 II.

4 Methods

5 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.

6 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 94ºC for 5 min (initiation), 35 cycles at 94ºC for 30s (denaturation), 60ºC (A66G MTRR) or 57ºC (T-34C CYP17) for 40s (annealing) and 72ºC 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 37ºC 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 37ºC 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.

7 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 ?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).

8 III.

9 Results

Were included eighty-two women in this study. Clinical-pathological features were available (Table 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).

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

IV.

11 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 **??** 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.

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.

12 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.

13 Funding

This work was supported by UESB and Fundação de Amparo à Pesquisa do Estado da Bahia (Fapesb).

1

Figure 1: Table 1

1

Figure 2: Table 1 :

2

Genotype or allele Frequency (%)	?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: ?2: chi-square. Statistically significant: p=0.05.

Figure 3: Table 2 :

3

Figure 4: Table 3 :

.1 Acknowledgment

JOC, VSS, JSO and SSO are fellow supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brazil).

[Mol Med Rep] , *Mol Med Rep* 16 (2) p. .

[Hu et al. ()] , J Hu , G W Zhou , N Wang , Y J Wang . 2010.

[Wilson et al. ()] 'A common variant in methionine synthase reductase combined with low cobalamin (vitamin B12) increases risk for spina bifida'. A Wilson , R Platt , Q Wu , D Leclerc , B Christensen , H Yang . *Mol Genet Metab* 1999. 67 (4) p. .

[Kato et al. ()] 'African American-preponderant single nucleotide polymorphisms (SNPs) and risk of breast cancer'. I Kato , M Cichon , C L Yee , S Land , J F Korczak . *Cancer Epidemiol* 2009. 33 (1) p. .

[Sun et al. ()] 'Association between CYP17 T-34C rs743572 and breast cancer risk'. J Sun , H Zhang , M Gao , Z Tang , D Guo , X Zhang . *Oncotarget* 2018. 9 (3) p. .

[Zhang et al. ()] 'Association of genetic polymorphisms of ER-alpha and the estradiolsynthesizing enzyme genes CYP17 and CYP19 with breast cancer risk in Chinese women'. L Zhang , L Gu , B Qian , X Hao , W Zhang , Q Wei . *Breast Cancer Res Treat* 2009. 114 (2) p. .

[Wang et al. ()] 'Association of MTRR A66G polimorphism with cancer susceptibility: Evidence from 85 studies'. P Wang , S Li , M Wang , J He , S Xi . *J Cancer* 2017. 8 (2) p. .

[Mo et al. ()] 'Associations between folate metabolism enzyme polymorphisms and breast cancer: A metaanalysis'. W Mo , Y Ding , Y Zheng , D Zou , X Ding . *Breast J* 2020. 26 (3) p. .

[Rojas and Stuckey ()] 'Breast Cancer Epidemiology and Risk Factors'. K Rojas , A Stuckey . *Clin Obstet Gynecol* 2016. 59 (4) p. .

[Low et al. ()] 'Breast cancer: The translation of big genomic data to cancer precision medicine'. S K Low , H Zembutsu , Y Nakamura . *Cancer Sci* 2018. 109 (3) p. .

[Schnitt ()] 'Classification and prognosis of invasive breast cancer: from morphology to molecular taxonomy'. S J Schnitt . *Modern Pathology* 2010. p. .

[Lilyquist et al. ()] 'Common Genetic Variation and Breast Cancer Risk-Past, Present, and Future'. J Lilyquist , K J Ruddy , C M Vachon , F J Couch . *Cancer Epidemiol Biomarkers Prev* 2018. 27 (4) p. .

[Kuligina et al. ()] 'CYP17 polymorphism in the groups of distinct breast cancer susceptibility: comparison of patients with the bilateral disease vs. monolateral breast cancer patients vs. middle-aged female controls vs. elderly tumor-free women'. E S Kuligina , A V Togo , E N Suspitsin , M Y Grigoriev , K M Pozharisskiy , O L Chagunava . *Cancer Lett* 2000. 156 (1) p. .

[Cribb et al. ()] 'CYP17, catechol-o-methyltransferase, and glutathione transferase M1 genetic polymorphisms, lifestyle factors, and breast cancer risk in women on Prince Edward Island'. A E Cribb , Joy Knight , M Guernsey , J Dryer , D Hender , K Shawwa , A . *Breast J* 2011. 17 (1) p. .

[Olteanu et al. ()] 'Differences in the Efficiency of Reductive Activation of Methionine Synthase and Exogenous Electron Acceptors between the Common Polymorphic Variants of Human Methionine Synthase Reductase'. H Olteanu , T Munson , R Banerjee . *Biochemistry* 2002. p. .

[Hasan et al. ()] 'Disturbed homocysteine metabolism is associated with cancer'. T Hasan , R Arora , A K Bansal , R Bhattacharya , G S Sharma , L R Singh . *Exp Mol Med* 2019. 51 (2) p. .

[Clemons and Goss ()] 'Estrogen and the risk of breast cancer'. M Clemons , P Goss . *The New England Journal of Medicine* 2001. p. .

[Chen and Pei ()] 'Factors influencing the association between CYP17 T34C polymorphism and the risk of breast cancer: meta-regression and subgroup analysis'. Y Chen , J Pei . *Breast Cancer Res Treat* 2010. 122 (2) p. .

[Hiraoka and Kagawa ()] 'Genetic polymorphisms and folate status'. M Hiraoka , Y Kagawa . *Congenit Anom (Kyoto)* 2017. 57 (5) p. .

[Shiovitz and Korde ()] 'Genetics of breast cancer: a topic in evolution'. S Shiovitz , L A Korde . *Ann Oncol* 2015. 26 (7) p. .

[Binia et al. ()] 'Geographical and ethnic distribution of single nucleotide polymorphisms within genes of the folate/homocysteine pathway metabolism'. A Binia , A V Contreras , S Canizales-Quinteros , V A Alonzo , M E Tejero , I Silva-Zolezzi . *Genes Nutr* 2014. 9 (5) p. 421.

[Apostolou and Fostira ()] 'Hereditary breast cancer: the era of new susceptibility genes'. P Apostolou , F Fostira . *Biomed Res Int* 2013. 2013. p. 747318.

[Abé-Sandes et al. ()] 'Heterogeneity of the Y chromosome in Afrobrazilian populations'. K Abé-Sandes , W A Silva Junior , M A Zago . *Human Biology* 2004. p. .

[Bottiglieri ()] 'Homocysteine and folate metabolism in depression'. T Bottiglieri . *Prog Neuropsychopharmacol Biol Psychiatry* 2005. 29 (7) p. .

206 [MTRR A66G polymorphism and breast cancer risk: a meta-analysis Breast Cancer Res Treat] 'MTRR A66G
207 polymorphism and breast cancer risk: a meta-analysis'. *Breast Cancer Res Treat* 124 (3) p. .

208 [Suzuki et al. ()] 'Onecarbon metabolism-related gene polymorphisms and risk of breast cancer'. T Suzuki , K
209 Matsuo , K Hirose , A Hiraki , T Kawase , M Watanabe . *Carcinogenesis* 2008. 29 (2) p. .

210 [Carey et al. ()] 'Polycystic ovaries and premature male pattern baldness are associated with one allele of the
211 steroid metabolism gene CYP17'. A H Carey , D Waterworth , K Patel , D White , J Little , P Novellm .
212 *Human Molecular Genetics* 1994. p. .

213 [Weiner et al. ()] 'Polymorphisms in the folate-metabolizing genes MTR, MTRR, and CBS and breast cancer
214 risk'. A S Weiner , U A Boyarskikh , E N Voronina , I A Selezneva , T V Sinkina , A F Lazarev . *Cancer*
215 *Epidemiol* 2012. 36 (2) p. .

216 [Guo et al. ()] 'Polymorphisms of estrogen-biosynthesis genes CYP17 and CYP19 may influence age at menarche:
217 a genetic association study in Caucasian females'. Y Guo , D H Xiong , T L Yang , Y F Guo , R R Recker ,
218 H W Deng . *Hum Mol Genet* 2006. 15 (16) p. .

219 [Syamala et al. ()] 'Possible risk modification by polymorphisms of estrogen metabolizing genes in familial breast
220 cancer susceptibility in an Indian population'. V S Syamala , V Syamala , V R Sheeja , R Kuttan , R
221 Balakrishnan , R Ankathil . *Cancer Invest* 2010. 28 (3) p. .

222 [Stratton and Rahman ()] 'The emerging landscape of breast cancer susceptibility'. M R Stratton , N Rahman .
223 *Nat Genet* 2008. 40 (1) p. .

224 [Wen et al. ()] *Unifying mechanism in the initiation of breast cancer by metabolism of estrogen*, C Wen , L Wu ,
225 L Fu , B Wang , H Zhou . 2017. (Review)