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MTHFR and *MTR* Polymorphisms and Breast Cancer in Brazilian Women

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***Abstract*— Breast cancer (BC) is the second most common cancer, and mortality rates remain high among Brazilian women. However, the role of single nucleotide polymorphisms (SNPs) in one-carbon metabolism genes in breast cancer in Brazilian women is less clear. We aimed examine the association between the SNPs, in two genes in one-carbon metabolism alone and in cumulation, and the risk of breast cancer in an Brazilian population based case-control study of 257 breast cancer cases and 177 controls. Our hypothesis was woman who carries more risk genotypes has a higher susceptibility for developing breast cancer. Genotyping for MTHFR C677T and MTR A2756G polymorphisms were performed using polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) method. Our results in population studied indicated that 677 C>T and 2756 A>G substitution does not appear to influence the risk of breast cancer. The cumulative effect was not observed with the OR being gradually elevated with increasing number of risk genotypes. However, larger studies are needed to further examine this interactions in this pathway and breast cancer risk in Brazilian women, as well in women of others nationalities.**

***Index Terms*— breast cancer, MTHFR C677T, MTR A2756G, one-carbon metabolism, polymorphisms.**

I. Introduction

Worldwide, breast cancer (BC) is the second most common cancer, the incidence rates vary widely across the world regions, nearly fourfold, and it is the most frequent cause of cancer death in women in less developed regions [1]. In Brazil, it is estimated 57,960 new cases for the year 2016 [2], and this disease is one of the main challenges faced by the Brazilian government, whose the mortality rate has progressively increased in recent years [3]

The disease is multifactorial, involving biological and endocrine factors, reproductive life, behavior and lifestyle [2], and the genetic risk factors can modify the risk of disease. Thus, alterations in the nucleotide sequences may be associated with cancer risk [4-6]. There are several single nucleotide polymorphisms (SNPs) in genes important in breast cancer, including the one-carbon metabolism pathway [7-10].

The methylenetetrahydrofolate reductase (MTHFR), one of the key enzymes in the one-carbon metabolism, catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate in 5-methyltetrahydrofolate [11, 12], while the enzyme methionine synthase (MTR), catalyzes the irreversible transfer of the methyl group of 5-methyltetrahydrofolate, promoting remethylation homocysteine to methionine [13].

The MTHFR 677 C>T and MTR *2756* A> G substitution have been considered to influence the enzymatic activity. Thence, studies have found association between the MTHFR C677T polymorphism [7, 14-20] and MTR A2756G polymorphism [7, 19, 21] in breast cancer, considered a genetic risk factor. Thus, reduced activity of these enzymes of one-carbon metabolism pathway could result in altered availability of methyl groups and impaired DNA methylation, and subsequently lead to cancer development [22].

The relationship between cumulative effect of genetic variants of MTHFR and MTR and breast cancer has not been extensively studied in the Brazilian population. Our hypothesis is that individual genetic variants when considered cumulatively can result in considerable effects. Thus, we aimed to examine the effect alone and in cumulation of MTHFR C677T and MTR A*2756*G polymorphism on the risk of breast cancer in Brazilian women.

II. Material and Methods

A. *Study population and data collection*

This hospital-based masked case-control study was developed at Odete Valadares Hospital in Belo Horizonte, Minas Gerais, Brazil, involving 257 breast cancer cases and 177 controls; more detail recruitment was described previously by our group [23, 24]. The research was approved by the National Committee of Ethics in Research, and all protocols used were approved and informed written consent for participation was gained from all patients.

B. *DNA extraction*

Genomic DNA was obtained from stored buffy coat. Briefly, buffy coats were digested using lysing solution,

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Madissen (0.1M Tris-HCl pH = 8.0; 0.4 M EDTA; 0.2% SDS; 1M NaCl; pH = 8.0), followed by addition of proteinase K (20 mg/mL) and incubated overnight at 37 °C. Then, the DNA was precipitated with saturated phenol and chloroform: isoamyl alcohol (24:1). Finally, cold isopropyl alcohol was added and homogenized slowly until the precipitation of DNA, which was dried at room temperature.

The extracted DNA was quantified in a spectrophotometer at 280nm and 260nm wavelength for evaluation of DNA purity and analyzed for integrity by comparing the bands obtained with the bands of different known concentrations of human DNA patterns by agarose gel electrophoresis 0.8%.

C. *Genotyping*

The polymorphisms of the genes under study were detected by the method restriction fragment length polymorphism (RFLP), after DNA amplification by polymerase chain reaction (PCR) (Table 1).

Table 1: Polymerase chain reaction and restriction protocols.

Gene	*Primer* (5'- 3')	PCR conditions	Restriction enzyme	Size of the fragment / Genotyping of patients
MTHFR C677T[a]	MTHFR677 f: TGA AGG AGA AGG TGT CTG CGG GA MTHFR677 r: AGG ACG GTG CGG TGA GAG TG	1 cycle 95°/5 min; 35 cycles to 55°/1 min, 72°/1 min, 95°/1 min; 1 cycle 72°/10 min.	Hinf I	198 bp CC: 198 bp TT: 175 e 23 bp CT: 198, 175 and 23 bp
MTR A2756G[b]	MTR2756 f: CAT GGA AGA ATA TGA AGA TAT TAG AC MTR2756 r: GAA CTA GAA GAC AGA AAT TCT CTA	1 cycle 92°/2 min; 35 cycles to 56°/1 min, 72°/1 min 30 seg, 92°/1 min; 1 cycle 56° C/1 min; 72° C/1 min and 30 seg; 10° C/10 min.	HaeIII	189 bp AA: 189 bp GG: 159 and 30 bp AG: 189, 159 e 30 bp

Abbreviations: bp = base pair; min= minutes
[a] FROSST et al [25]
[b] LECLERC et al [26]

D. *Statistical Analysis*

Descriptive variables were compared between number of risk genotype using chi-square test for categorical variables and Kruskal-Wallis test for continuous variable. The association between MTHFR and MTR genotype, and breast cancer were assessed by logistical regression model with results expressed as Odds Ratios (ORs) and their 95% confidence intervals (CIs) after adjusting for age and menopausal status. Hardy–Weinberg Equilibrium was tested to compare the observed with expected genotype frequencies. Analyses were performed by using SPSS® software, version 20 (SPSS INC. Chicago, IL. USA). All P-values were two sided, and a P-value <0.05 was considered statistically significant.

III. Results

Characteristics of the study population are shown in Table 2. Overall, women did not differ in function of the number of risk genotypes, suggesting homogeneity of the sample. A total of 257 breast cancer cases and 177 controls were included in final analyses.

The results of the selected SNPs in one-carbon metabolism genes and the breast cancer risk were shown in table 3. The MTH A2756G was associated with breast cancer, with decreased risk, in the crude analyses (OR= 0.551, 95% CI: 0.335 – 0.908). After adjustment for age and menopausal status the significance statistical was lost (OR= 0.602, 95% CI: 0.335 – 1.028). We did not observe any difference between increase of the number of risk genotypes and breast cancer risk, indicating that the SNPs of one-carbon metabolism genes evaluated not associated with this disease in Brazilian women included in this study.

Table 2: Characteristics of participants included in the study.

Variables	Number of risk genotypes			
	0/2 (n=163)	1/2 (n=184)	2/2 (n=39)	p-value
Age (mean ± SD)	51.44 ± 11.18	52.25 ± 12.07	54.9 ± 10.98	0.151[#]
Age of menarche				
≥ 13	99	108	27	0.514*
< 13	62	74	12	
Menopausal Status				
premenopausal	68	69	11	0.277*
postmenopausal	94	112	28	
Family history of BC				
No	134	146	32	0.988*
Yes	29	33	7	

[#]The Kruskal-Wallis test;*The chi-square test.

Table 3: Associations between MTHFR and MTR single nucleotide polymorphisms (SNPs) and breast cancer in Brazilian women.

	Case [n (%)]	Control [n (%)]	*OR* (CI 95%)[a]	*OR* (CI 95%)[b]
MTHFRC677T[*]				
CC	145 (56.6 %)	98 (55.4 %)	1	1
CT+TT	111 (43.4 %)	79 (44.6 %)	1.053 (0.716 – 1.549)	0.979 (0.956 – 1.003)
MTH A2756G[**]				
AA	165 (71.4 %)	127 (81.9 %)	1	1
AG+GG	66 (28,6 %)	28 (18.1)	**0.551 (0.335 – 0.908)**	0.602 (0.335 – 1.028)

Results by logistic regression [a]Crude, [b]Adjusted for age and menopausal status. [*]MTHFRC677T information was available: 256 (99.61%) breast cancer cases and 177 (100%) controls. [**] MTH A2756G information was available: 231 (89.88%) breast cancer cases and 155 (87.57%) controls.

Table 4: Cumulative effect of SNPs susceptibility for breast cancer.

Number of risk genotype[a]	Case [n (%)]	Control [n (%)]	*OR* (IC 95%)*	p-value
0/2	88 (38,1)	75 (48,4)	1	
1/2	119 (51,5)	65 (41,9)	1.116 (0.519 – 2.399)	0.780
2/2	24 (10,4)	15 (9,7)	0.694 (0.323 – 1.493)	0.351

[a]Based on the two SNPs. *Data were calculated by logistic regression adjusted for age and menopausal status.

IV. Discussion

The results from this study demonstrated that family history does not appear to have an association with increasing the number of risk genotypes in SNPs of one-carbon

metabolism. These data suggest that women included in the study did not differ in general characteristics. Recent study concluded that clinical management should not differ between women with and without family history, because the authors not find evidence to support an association between family history of breast cancer and severity and breast cancer-specific mortality [27].

Results of studies with women from different populations are still inconclusive. Some found an association between MTHFR C677T variant alleles and increased risk for breast cancer [14-16, 18]; while others detected a reduced risk [28]; while others found no significant relationship [29].

Polymorphism studies in MTR A2756G in relation to risk of breast cancer have reported mixed results, including no association [30-33], inverse relationship [10, 34] or positive association [19, 21] with the variant allele.

Hence, each of these variants was found to be independently and moderately associated with breast cancer risk in different populations, thus, the combinations of risk alleles could be a cumulative effect on breast cancer, and we tested this hypothesis in Brazilian women.

In this study, no significant associations were detected between breast cancer risk and SNPs evaluated, as well the cumulative effect of these SNPs by counting the number of genotypes associated with breast cancer risk. In breast cancer [35] and other types of cancer, patients carrying higher number of risk genotypes shown increased risk for colorectal cancer [36], gastric carcinoma [37], and thyroid cancer [38].

Recently, models have been developed to predict the risk of breast cancer in women. These models may consider the SNP-SNP interactions in breast cancer [39] as well as genetic variants and established risk factors [40].

One point worth mentioning is the effect of ethnic in the associations of these SNPs described in the literature, the data vary greatly depending on the population, in which the study is conducted, suggesting that these SNPs may have different effects in different populations. This fact can justify incidence rates varying widely across the world regions. Similar studies can be conducted in different ethnic groups and populations to provide more insights into the molecular pathophysiology of breast cancer.

V. Conclusion

Taken together, in population studied, the MTHFR 677 C>T and MTR 2756 A> G substitution does not appear to influence the risk of breast cancer, in Brazilian women.

Similar studies can be conducted in different ethnic groups and populations to provide more insights into the molecular pathophysiology of breast cancer.

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