

MTHFR and MTR Polymorphisms and Breast Cancer in Brazilian Women

Lisiane Lopes da Conceição, Milene Cristine Pessoa, Helen Hermana Miranda Hermsdorff, Renata Nascimento de Freitas, Maria do Carmo Gouveia Peluzio

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

Lisiane Lopes da Conceição, MsC, Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, zip code: 36571-900,

Milene Cristine Pessoa, PhD, Departament of Clinical and Social Nutrition, Universidade Federal de Ouro Preto, Minas Gerais, zip code: 35400-000, Brazil.

Helen Hermana Miranda Hermsdorff, PhD, Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, zip code: 36571-900, Brazil.

Renata Nascimento de Freitas, PhD, Center of Biological Sciences Reseach, Universidade Federal de Ouro Preto, Minas Gerais, zip code: 35400-000, Brazil.

Maria do Carmo Gouveia Peluzio, PhD, Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, zip code: 36571-900, Brazil.

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 A2756G 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,

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 | MTHFR677 F TGA AGG AGA AGG TGT CTG | 1 cycle 95°/5 min; | Hinf I | |
| C677T ^a | CGG GA | 35 cycles to 55°/1 min; | | 198 bp |
| | MTHFR677 r. AGG ACG GTG CGG TGA GAG | 72°/1 min, 95°/1 min; | | CC: 198 bp |
| | TG | 1 cycle 72°/10 min. | | TT: 175 e 23 bp |
| | | | | CT: 198, 175 and 23 bp |
| MTR | MTR2756 F CAT GGA AGA ATA TGA AGA | 1 cycle 92°/2 min; | HaeIII | |
| A2756G ^b | TAT TAG AC | 35 cycles to 56°/1 min; | | 189 bp |
| | MTR2756 r. GAA CTA GAA GAC AGA AAT | 72°/1 min 30 seg, 92°/1 min; | | AA: 189 bp |
| | TCT CTA | 1 cycle 56° C/1 min; | | GG: 159 and 30 bp |
| | | 72° C/1 min and 30 seg; 10° C/10 min. | | AG: 189, 159 e 30 bp |

Abbreviations: bp = base pair; min= minutes

^aFROSST et al [25]

^bLECLERC 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 | | | p-value |
|----------------------|--------------------------|----------------|---------------|--------------------|
| | 0/2 (n=163) | 1/2 (n=184) | 2/2 (n=39) | |
| 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 |
|----------------------|-----------------|--------------------|--------------------------|--------------------------|
| <i>MTHFR C677T</i> | | | | |
| 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) |
| <i>MTH A2756G</i> ** | | | | |
| 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 ^aCrude, ^bAdjusted for age and menopausal status. ^{*}MTHFR C677T 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%) ^a | 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 |

^aBased 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.

ACKNOWLEDGMENT

THE AUTHORS THANK THE COORDENAÇÃO DE APERFEIÇOAMENTO DE PESSOAL DE NÍVEL SUPERIOR (CAPES) BRASÍLIA, BRAZIL, CONSELHO NACIONAL DE DESENVOLVIMENTO CIENTÍFICO E TECNOLÓGICO (CNPQ) BRASÍLIA, BRAZIL, AND FUNDAÇÃO DE AMPARO À PESQUISA DE MINAS GERAIS (FAPEMIG) BELO HORIZONTE, BRAZIL

FOR THEIR FINANCIAL SUPPORT. M.C.G.P. IS CNPQ FELLOWS. L.L.C. IS THE RECIPIENT OF CAPES GRANT.

REFERENCES

- [1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Internacional Journal of Cancer*. 2015;136(5):E359-E86.
- [2] INCA. Ministério da Saúde. Estimativa 2016: Incidência de câncer no Brasil. Rio de Janeiro: INCA, 2015. p. 122.
- [3] Cecilio AP, Takakura ET, Jumes JJ, dos Santos JW, Herrera AC, Victorino VJ, et al. Breast cancer in Brazil: epidemiology and treatment challenges. *Breast Cancer: Targets and Therapy*. 2015;(7):43-49.
- [4] Xie S-Z, Liu Z-Z, Yu J-h, Liu L, Wang W, Xie D-L, et al. Association between the MTHFR C677T polymorphism and risk of cancer: evidence from 446 case-control studies. *Tumor Biology*. 2015;36(11):8953-8972.
- [5] Tang M, Wang S-Q, Liu B-J, Cao Q, Li B-J, Li P-C, et al. The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and tumor risk: evidence from 134 case-control studies. *Molecular Biology Reports* 2014;41(7):4659-4673.
- [6] Yu K, Zhang J, Zhang J, Dou C, Gu S, Xie Y, et al. Methionine synthase A2756G polymorphism and cancer risk: a meta-analysis. *European Journal of Human Genetics*. 2010;18(3):370-378.
- [7] Jiang-Hua Q, De-Chuang J, Zhen-Cuo L, Shu-de C, Zhenzhen L. Association of methylenetetrahydrofolate reductase and methionine synthase polymorphisms with breast cancer risk and interaction with folate, vitamin B6, and vitamin B12 intakes. *Tumor Biology*. 2014;35(12):11895-11901.
- [8] Kumar P, Yadav U, Rai V. Methylenetetrahydrofolate reductase gene C677T polymorphism and breast cancer risk: Evidence for genetic susceptibility. *Meta Gene*. 2015;6:72-84.
- [9] Macis D, Maisonneuve P, Johansson H, Bonanni B, Botteri E, Iodice S, et al. Methylenetetrahydrofolate reductase (MTHFR) and breast cancer risk: a nested-case-control study and a pooled meta-analysis. *Breast Cancer Research and Treatment*. 2007;106(2):263-271.
- [10] Lu M, Wang F, Qiu J. Methionine synthase A2756G polymorphism and breast cancer risk: a meta-analysis involving 18,953 subjects. *Breast Cancer Research and Treatment*. 2010;123(1):213-217.
- [11] Bailey LB, Gregory JF. Polymorphisms of Methylenetetrahydrofolate Reductase and Other Enzymes: Metabolic Significance, Risks and Impact on Folate Requirement. *The Journal of Nutrition*. 1999;129(5): 919-22.
- [12] Izmirli M. A literature review of MTHFR (C677T and A1298C polymorphisms) and cancer risk. *Molecular Biology Reports*. 2013;40(1):625-637.
- [13] Jiménez-Chillarón JC, Díaz R, Martínez D, Pentinat T, Ramón-Krauel M, Ribó S, et al. The role of nutrition on epigenetic modifications and their implications on health. *Biochimie* 2012;94(11):2242-2263.
- [14] Gao C-M, Tang J-H, Cao H-X, Ding J-H, Wu J-Z, Jie Wang J, et al. MTHFR polymorphisms, dietary folate intake and breast cancer risk in Chinese women. *Journal of Human Genetics* 2009;54(7):414-418.
- [15] Wu X-Y, Ni J, Xu W-J, Zhou T, Wang X. Interactions Between MTHFR C677T - A1298C Variants and Folic Acid Deficiency Affect Breast Cancer Risk in a Chinese Population. *Asian Pacific Journal of Cancer Prevention*. 2012;13(5):2199-2206.
- [16] Stevens VL, McCullough ML, Pavluck AL, Talbot JT, Feigelson HS, Thun MJ, et al. Association of Polymorphisms in One-Carbon Metabolism Genes and Postmenopausal Breast Cancer Incidence. *Cancer Epidemiology Biomarkers and Prevention* 2007;16(6):1140-1147.
- [17] Qi X, Ma X, Yang X, Fan L, Zhang Y, Zhang F, et al. Methylenetetrahydrofolate reductase polymorphism and breast cancer risk: a meta-analysis from 41 studies with 16,480 cases and 22,388 controls. *Breast Cancer Research and Treatment* 2010;123(2):499-506.
- [18] Ozen F, Erdis E, Sik E, Silan F, Uludag A, Ozdemir O. Germ-line MTHFR C677T, FV H1299R and PAI-1 5G/4G Variations in Breast Carcinoma. *Asian Pacific Journal of Cancer Prevention*. 2013;14(5):2903-2908.
- [19] Barbosa RCC, M CD, Cordeiro DE, Vieira AP, Rabenhorst SH. Interaction of MTHFR C677T and A1298C, and MTR A2756G gene polymorphisms in breast cancer risk in a population in Northeast Brazil. *Anticancer Research* 2012;32(11):4805-4811.
- [20] Awwad N, Yousef A-M, Abuhaliema A, Abdalla I, Yousef M. Relationship between Genetic Polymorphisms in MTHFR (C677T, A1298C and their Haplotypes) and the Incidence Of Breast Cancer

- among Jordanian Females - Case-Control Study. *Asian Pacific Journal of Cancer Prevention*. 2015;16(12):5007-5011.
- [21] Hosseini M. Role of polymorphism of methyltetrahydrofolate homocysteine methyl transferase (MTR) A2756G and breast cancer risk. *Polish Journal of Pathology*. 2013;64(3):191-195.
- [22] Gong Z, Yao S, Zirpoli G, David Cheng T-Y, Roberts M, Khoury T, et al. Genetic variants in one-carbon metabolism genes and breast cancer risk in European American and African American women. *International Journal of Cancer*. 2015;137(3):666-677.
- [23] Abranches MV, Mendes MCS, Pena GG, Maia YCP, Ribeiro SMR, Franceschini SCC, et al. Antioxidant vitamins and cytokines are altered in breast cancer. *European Journal of Cancer Prevention*. 2011;20(5):403-410.
- [24] Pena GG, Maia YCP, Mendes MCS, Furtado WR, Machado-Coelho GLL, Freitas RN. Physical Activity Is Associated with Malignant and Benign Breast Diseases in Low-Income Brazilian Women. *Nutrition and Cancer*. 2013;66(4):707-715.
- [25] Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nature Genetics* 1995;10(1):111-113.
- [26] Leclerc D, Campeau E, Goyette P, Adjalla CE, Christensen B, Ross M, et al. Human methionine synthase: cDNA cloning and identification of mutations in patients of the cblG complementation group of folate/cobalamin disorders. *Human Molecular Genetics*. 1996; 5(12):1867-1874.
- [27] Melvin JC, Wulaningsih W, Hana Z, Purushotham AD, Pinder SE, Fentiman I, et al. Family history of breast cancer and its association with disease severity and mortality. *Cancer Medicine*. 2016; 5(5):942-949.
- [28] Chou Y-C, Wu M-H, Yu J-C, Lee M-S, Yang T, Shih H-L, et al. Genetic polymorphisms of the methylenetetrahydrofolate reductase gene, plasma folate levels and breast cancer susceptibility: a case-control study in Taiwan. *Carcinogenesis*. 2006;27(11):2295-2300.
- [29] Shrubsole MJ, Gao Y-T, Cai Q, Shu XO, Dai Q, Hébert JR, et al. MTHFR Polymorphisms, Dietary Folate Intake, and Breast Cancer Risk: Results from the Shanghai Breast Cancer Study. *Cancer Epidemiology, Biomarkers and Prevention*. 2004;13(2): 190-196.
- [30] Shrubsole MJ, Gao Y-T, Cai Q, Shu XO, Dai Q, Jin F, et al. MTR and MTRR Polymorphisms, Dietary Intake, and Breast Cancer Risk. *Cancer Epidemiology, Biomarkers and Prevention* 2006;15(3):586-8.
- [31] Zhong S, Xu J, Li W, Chen Z, Ma T, Zhao J. Methionine synthase A2756G polymorphism and breast cancer risk: An up-to-date meta-analysis. *Gene*. 2013;527(2):510-515.
- [32] Tao MH, Shields PG, Nie J, Marian C, Ambrosone CB, McCann SE, et al. DNA promoter methylation in breast tumors: No association with genetic polymorphisms in MTHFR and MTR. *Cancer Epidemiology Biomarkers and Prevention*. 2009;18(3): 998-1002.
- [33] Weiner AS, Boyarskikh UA, Voronina EN, Selezneva IA, Sinkina TV, Lazarev AF, et al. Polymorphisms in the folate-metabolizing genes MTR, MTRR, and CBS and breast cancer risk. *Cancer Epidemiology*. 2012;36(2):e95-e100.
- [34] Kotsopoulos J, Zhang WW, Zhang S, McCready D, Trudeau M, Zhang P, et al. Polymorphisms in folate metabolizing enzymes and transport proteins and the risk of breast cancer. *Breast Cancer Research and Treatment*. 2008;112(3):585-593.
- [35] Sun M-Y, Du H-Y, Zhu A-N, Liang H-Y, de Garibay G, Li F-X, et al. Genetic Polymorphisms in Estrogen-Related Genes and the Risk of Breast Cancer among Han Chinese Women. *International Journal of Molecular Sciences*. 2015;16(2):4121-4135.
- [36] Xiong F, Wu C, Bi X, Yu D, Huang L, Xu J, et al. Risk of Genome-Wide Association Study-Identified Genetic Variants for Colorectal Cancer in a Chinese Population. *Cancer Epidemiology Biomarkers & Prevention*. 2010;19(7):1855-1861.
- [37] Machado JC, Figueiredo C, Canedo P, Pharoah P, Carvalho R, Nabais S, et al. A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. *Gastroenterology*. 2003;125(2):364-371.
- [38] Figlioli G, Chen B, Elisei R, Romei C, Campo C, Cipollini M, et al. Novel genetic variants in differentiated thyroid cancer and assessment of the cumulative risk. *Scientific Reports*. 2015;5:8922.
- [39] Chuang LY, Lin MC, Chang HW, Yang CH, editors. Odds Ratio-Based Genetic Algorithm for Prediction of SNP-SNP Interactions in Breast Cancer Association Study. *Advanced Information Networking and Applications Workshops (WAINA), 2012 26th International Conference on; 2012 26-29 March 2012*
- [40] Lee CPL, Irwanto A, Salim A, Yuan J-m, Liu J, Koh WP, et al. Breast cancer risk assessment using genetic variants and risk factors in a Singapore Chinese population. *Breast Cancer Research*. 2014;16(3):1-13.

Lisiane Lopes da Conceição, MSc, Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, zip code: 36571-900, Brazil.

Milene Cristine Pessoa, PhD, Department of Clinical and Social Nutrition, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, zip code: 35400-000, Brazil

Helen Hermana Miranda Hermsdorff, PhD, Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, zip code: 36571-900, Brazil.

Renata Nascimento de Freitas, PhD, Center of Biological Sciences Reseach - NUPEB, Molecular Epidemiology Laboratory, Universidade Federal de Ouro Preto, Ouro Preto, Minas Gerais, zip code: 35400-000, Brazil.

Maria do Carmo Gouveia Peluzio, PhD, Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, Minas Gerais, zip code: 36571-900, Brazil.