Breast Cancer Res Treat (2009) 115:431–432
DOI 10.1007/s10549-008-0054-x

LETTER TO THE EDITOR

Dihydrofolate reductase (DHRF) 19-bp intron-1 deletion and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms in breast cancer

Ragip Çam · Aydan Eroglu · Yonca Egin · Nejat Akar

Received: 1 May 2008 / Accepted: 1 May 2008 / Published online: 24 May 2008

The causes of breast cancer (BC) are not clearly known, although different genetic and environmental factors play role in its development. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate-mediated one-carbon metabolism, regulating the DNA methylation and synthesis. Reduced MTHFR activity is associated with genomic DNA hypomethylation. MTHFR is suspected to play a role in the etiology of cancer, via its effects on DNA methylation and nucleotide synthesis [1]. The C to T substitution at nucleotide 677 of MTHFR gene converts an alanine to a valine is associated with reduced enzyme activity and causes increased plasma homocysteine levels. Associations of MTHFR C677T polymorphism with BC have been investigated [1–4].

Dihydrofolate reductase (DHFR) is also important folate metabolizing enzyme that is essential for DNA synthesis and homocysteine remethylation. Recently, Johnson et al. described a 19-bp deletion polymorphism in intron-1 of DHFR that might affect gene expression [5]. More recently, Gellekink et al. reported that 19-bp del/del genotype was associated with a lower plasma homocysteine level and suggested that 19-bp deletion might increase DHFR expression thereby facilitating homocysteine remethylation [6].

The aim of the present study was to evaluate the association of BC risk with MTHFR C677T and DHFR 19-bp deletion polymorphisms.

R. Çam · A. Eroglu (✉)
Department of General Surgery, Ankara University Medical School, Cebeci Kampus, Ankara 06590, Turkey
e-mail: aydaneroglu@hotmail.com

Y. Egin · N. Akar
Department of Pediatric Molecular Genetic, Ankara University Medical School, Cebeci Kampus, Ankara 06590, Turkey

The study consisted of 110 women with BC. Ninety-five healthy women (in matched control) were used as control. Genomic DNA isolation was performed from peripheral venous blood by standard phenol–chloroform extraction, and polymerase chain reaction of 19-bp deletion polymorphism in intron-1 of DHFR was performed according to previously described method [5], using the following primers: F 5′-CCACGGTCGGGGTACCTGGG-3′, F 5′-ACGGTCGGGGTGGCCGACTC-3′ and R 5′-AAAAGGG GAATCCAGTCGG-3′. Amplification was made as follows: initial denaturation of one min at 94°C; followed by 35 cycles of 94°C/55 s, 64°C/55 s, 72°C/55 s, and final extension of 12 min at 72°C (Biometra, Germany). The resulting PCR products were 96-bp and 113-bp. Amplified products were subjected to 4% agarose gel. Genotype data were obtained from 179 subjects. MTHFR C677T polymorphism was determined by using commercially available LightCycler kit (Roche Diagnostic, Roche Molecular Biochemicals, Mannheim, Germany). Genotype data were obtained from 205 cases.

The groups were compared using χ^2 test and P-value < 0.05 was considered significant.

No differences were observed in the distribution of MTHFR C677T genotype or allele frequencies in the BC cases versus control subjects ($P > 0.05$; Table 1). A slightly increased frequency of TT genotype in cancer patients was found but this did not reach statistical significance. We did not find a significant association of DHFR 19-bp deletion polymorphism with BC risk. The frequency of 19-bp deletion allele was lower in BC patients than in control group.

Various groups have evaluated the association between MTHFR C677T polymorphism and BC risk, though the findings were conflicting. Some authors observed an increased risk for the carriers of TT genotype. Recent

Table 1 Distribution of MTHFR C677T and DHFR 19-bp intron deletion genotypes and allelic frequencies in breast cancer cases and control subjects

Genotype	Case subjects (%)	Control subjects	*P*-value
MTHFR 677CT	($n = 110$)	($n = 95$)	
CC	48 (43.6%)	47 (49.5%)	
CT	49 (44.5%)	42 (44.2%)	0.36
TT	13 (11.9%)	6 (6.3%)	
C allele	0.66	0.72	0.35
T allele	0.34	0.28	0.35
DHFR 19-bp deletion	($n = 79$)	($n = 95$)	
WT/WT	44 (55.7%)	38 (40%)	
WT/del	27 (34.2%)	47 (49.5%)	0.099
del/del	8 (10.1%)	10 (10.5%)	
WT allele	0.73	0.65	0.25
Del allele	0.27	0.35	0.25

WT, wild-type allele; del, deletion allele

meta-analysis by Macus et al. showed an association of TT genotype with increased BC risk in premenopausal women [4]. Although not statistically significant, we found a trend for higher MTHFR 677TT genotype (11.9%) and T allele frequency (0.34) than in control subjects (6.3%, 0.28, respectively). Our data support the hypothesis that the MTHFR polymorphism may influence the BC risk.

To our knowledge, this is the second study which has evaluated the possible correlation between DHFR 19-bp deletion polymorphism with BC risk. We observed a lower frequency of DHFR 19-bp deletion allele in breast cancer cases compared to control subjects (0.27 vs. 0.35) but it did not achieve statistically significance. Recently, Xu et al. have evaluated an association of this polymorphism with BC risk [7]. Our early results for DHFR are consistent with previous study involving no role of this polymorphism with BC risk.

Although we did not determine plasma homocysteine levels, folate concentration and dietary folate intake in our cases, our early results showed that neither of MTHFR 677CT and DHFR 19-bp deletion polymorphisms were associated with BC risk. However further studies with larger series are necessary to reach final conclusion.

References

1. Suzuki T, Matsuo K, Hirose K, Hiraki A, Kawase T, Watanabe M, Yamashita T, Iwata H, Tajima K (2008) One-carbon metabolism-related gene polymorphisms and risk of breast cancer. Carcinogenesis 29:356–362
2. Jakubowska A, Gronwald J, Menkiszak J et al (2007) Methylenetetrahydrofolate reductase polymorphisms modify BRCA1-associated with breast and ovarian cancer risk. Breast Cancer Res Treat 104:299–308
3. Martin DN, Boersma BJ, Howe TM, Goodman JE, Mechanic LE, Chanock SJ, Ambs S (2006) Association of MTHFR gene polymorphisms with breast cancer survival. BMC Cancer 6:257
4. Macis D, Maisonneuve P, Johansson H et al (2007) Methylenetetrahydrofolate reductase (MTHFR) and breast cancer risk: a nested-case–control study and a pooled meta-analysis. Breast Cancer Res Treat 106:263–271
5. Johnson WG, Stenroos ES, Spychala JR, Chatkupt S, Ming SX, Buyske S (2004) New 19 bp deletion polymorphism in intron-1 of dihydrofolate reductase (DHFR): a risk factor for spina bifida acting in mothers during pregnancy? Am J Med Genet 124A:339–345
6. Gellekink H, Blom HJ, van der Linden IJM, den Heijer M (2007) Molecular genetic analysis of the human dihydrofolate reductase gene: relation with plasma total homocysteine, serum and red blood cell folate levels. Eur J Hum Genet 15:103–109
7. Xu X, Gammon MD, Wetmur JG, Rao M, Gaudet MM, Teitelbaum SL, Britton JA, Neugut AI, Santella RM, Chen J (2007) A functional 19-base pair deletion polymorphism of dihydrofolate reductase (DHFR) and risk of breast cancer in multivitamin users. Am J Clin Nutr 85:1098–1102