MTHFR polymorphisms and breast cancer risk

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Submitted: 7 July 2010
Accepted: 17 August 2010

Arch Med Sci 2011; 7, 1: 134-137
DOI: 10.5114/aoms.2011.20618
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Abstract

Introduction: Two functional single nucleotide polymorphisms (SNPs) in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, C677T and A1298C, lead to decreased enzyme activity and affect chemosensitivity of tumor cells.

Material and methods: We evaluated these two common polymorphisms and breast cancer risk association in an Iranian sporadic breast cancer population-based case-control study of 294 breast cancer cases and 306 controls using a PCR-RFLP-based assay.

Results: Analyses of affected and controls show that homozygote genotype MTHFR 677CC has the highest frequency in both groups (28.3% in patients and 25.3% in control group). Genotype MTHFR 677CT and genotype MTHFR 1298AC were found to be statistically significant risk factors in our population (odds ratio: 1.6, 95% CI: 1.019-2.513, p = 0.041; and odds ratio: 2.575, 95% CI: 1.590-4.158, p = 0.001 respectively).

Conclusions: We can conclude based on the results of our study that a significant association between breast cancer and C677T and A1298C polymorphism might exist.

Key words: MTHFR gene, polymorphism, breast cancer, PCR-RFLP, susceptibility factor.

Introduction

MTHFR is a key enzyme in the folate metabolism pathway and regulates the intracellular folate pool for synthesis and methylation of DNA [1, 2]. Two common allele variants of the MTHFR gene have been described, C677T and A1298C, that lead to amino acid substitutions, Ala222Val and Glu429Ala, and to decreased enzyme activity [3-5].

Folate is involved in DNA methylation, synthesis, and repair. Low intake of folate may increase the risk of several cancers, including breast cancer [5, 6].

The enzyme methylenetetrahydrofolate reductase (MTHFR) irreversibly catalyzes 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the donor for the remethylation of homocysteine to methionine, the precursor for the universal methyl donor S-adenosylmethionine [7, 8]. Two common polymorphisms in the MTHFR gene have been characterized [9, 10]: the 677C → T [9, 11] and the 1298A → C polymorphism codes [10]. Individuals homozygous for the 1298C allele have approximately the same enzyme activity as those heterozygous for the 677T allele [10, 11].

We report here that the allele frequencies of MTHFR 677 and 1298 were significant in breast cancer patients in Iran.
Material and methods

Patient data

Studies were conducted on 294 carcinoma breast cancer patients treated with chemotherapy and 306 controls comprising postmenopausal women genotyped for MTHFR, aged 45-55 years. All patients were from the chemotherapy ward in the Special Medical Centre, Tehran, Iran. This study was ethically approved by the local Ethical Committee of Islamic Azad University from the point of view of patients’ and controls’ rights. A questionnaire and a consent form including questions on breast cancer risk factors were completed by each patient. The blood samples were collected from patients and controls prior to the start of treatment. Subjects were genotyped for MTHFR SNPS using genomic DNA extracted from peripheral blood lymphocytes. DNA was isolated from peripheral blood using a FlexiGene DNA extraction kit (Qiagen Germany).

Genotyping

The polymorphisms were detected using a modified PCR-RFLP method [12, 13, 16]. The PCR primers were synthesized by TAG Copenhagen A/S Primers which were:

1) A1298C polymorphism (256 bp),
   forward: 5´-CTTCTACCTGAAGAGCAATG-3´,
   reverse: 5´-CATGTCCACACGATGGAG-3´.

   The cycling conditions were 94°C, 30 min; 61°C, 30 min (35 cycles); 72°C, 60 min. The PCR products were digested with 1 unit of MboII (Figure 1).

2) C677T (183 bp),
   forward primer: GACCTGAAAGACTTGAAGGA,
   reverse primer: CGAGCTTATGGCTCTCG.

   The cycling conditions were 94°C, 30 min; 61°C, 30 min (35 cycles); 72°C, 60 min. The PCR products were digested with 1 unit of HinfI, and separated on a 4% agarose gel (Figure 2).

This method is able to detect all three possible genotypes for the polymorphism: homozygous wild type, heterozygous variant type and homozygous variant type.

The genotypes and allelic frequencies of MTHFR polymorphisms in patient and control groups were analysed by $\chi^2$ and Fisher’s exact tests. $P$ values < 0.05 were considered significant.

Results

There was a significant result for MTHFR 1298 and 677 polymorphism in relation to breast cancer risk. Analyses of affected and controls show that homozygote genotype MTHFR 677CC has the highest frequency in both groups (28.3% in patients and 25.3% in control group), $p = 0.001$.

On the other hand, the homozygous genotype MTHFR 1298 CC was more increased in the patient group (27.3%) compared with controls (17.7%), $p = 0.001$ (Tables I, II and Figure 3).

The genotype MTHFR 677 CT and genotype MTHFR 1298 AC were found or appeared to be important risk factors in our population (odds ratio: 1.6, 95% CI: 1.019-2.513, $p = 0.041$; and odds ratio: 2.575, 95% CI: 1.590-4.158, $p = 0.001$ respectively), while MTHFR 677 CC did not show any statistical significance (odds ratio: 1.2, $p = 0.334$).

Of course, MTHFR 1298 CC (27.3%) and MTHFR 677 CC (28.3%) have the highest frequency compared with MTHFR 677 and MTHFR 1298 polymorphism.

In our study there was an association between C677T and A1298C polymorphism and breast cancer risk.

We conclude that not only was 1298 CC associated with increased risk for breast cancer but also there is a relation between the presence of 677 CC and increased breast cancer risk.
Table I. MTHFR 677 and 1298 genotype frequencies [n (%)] for patients and controls: Analyses of 294 affected women and 306 controls show the highest frequency for C/C MTHFR 677 genotype (28.3 and 25.3 respectively) and C/C MTHFR 1298 genotype (27.3 and 17.7 respectively)

| Genotype | Patients n (%) | Controls n (%) | Total n (%) |
|----------|---------------|---------------|-------------|
| n        | 294           | 306           | 600         |
| MTHFR (677) |             |               |             |
| CC       | 168 (28.3)    | 150 (25.3)    | 318 (53.5)  |
| CT       | 84 (14.1)     | 90 (15.2)     | 174 (29.3)  |
| TT       | 42 (7.1)      | 60 (10.1)     | 102 (17.2)  |
| MTHFR (1298) |           |               |             |
| CC       | 162 (27.3)    | 105 (17.7)    | 267 (44.9)  |
| AC       | 96 (16.2)     | 135 (22.7)    | 231 (38.9)  |
| AA       | 36 (6.1)      | 60 (10.1)     | 96 (16.2)   |

Discussion

Martin DN [14] found that the MTHFR SNPs C677T and A1298C were associated with breast cancer survival.

Jakubowska found that MTHFR_677_C > T was associated with an increased risk while 1298_A > C polymorphism was associated with a decreased risk for breast and ovarian cancer. It appears that functional polymorphisms in the MTHFR gene modify the risk of breast cancer and may potentially alter the risk of ovarian cancer in women with an inherited predisposition [15].

Shrubsole MJ did not observe any effect of A1298C genotypes on breast cancer risk. He suggests that the MTHFR C677T polymorphisms may modify the association between dietary folate intake and breast cancer risk [16, 17].

However, other studies on colorectal cancer [18], colorectal adenoma [19-21], gastric cancer [22], lung cancer [23], and acute myeloid leukaemia [24] did not find any association or an increased risk of cancer for individuals with the TT genotype.

The C677T polymorphism has been examined in relation to several cancers [6, 22]. Many studies have also examined the correlation between MTHFR 677TT and breast cancer risk [18-20, 25-28].

In the first study in Jewish women, there was no significant difference of MTHFR C677T genotype between sporadic cases and controls [26].

In Caucasian women it was reported that the MTHFR 677 TT allele was more prevalent in cases than controls [27], while in other studies the reported risk for breast cancer was associated with both the C677T and A1298C polymorphisms [28].

In our study, a statistically significant association between MTHFR genotype and breast cancer risk was found. Therefore, we can conclude that there might be a relation between the presence of MTHFR 1298AA and 677CC genotype and increasing risk of breast cancer, whereas there was a decreased frequency for MTHFR 677 CT, TT and 1298 CT, AA compared with controls.

Acknowledgments

We thank all the patients for their kind collaboration and also the Islamic Azad University

Table II. Comparison between genotypes, odds ratio and p value showed that p value of genotype MTHFR 677 CT was the most important risk factor in our population; TC odds ratio, 1.6 (95% confidence interval; CI, 1.019-2.513), p = 0.041, CC odds ratio, 1.2 (95% CI, 0.829-1.737), p = 0.334, TT odds ratio, 1.333 (95% CI, 0.814-2.185), p = 0.253. Genotype MTHFR 1298 AC was the most important risk factor in our population; AC odds ratio, 2.571 (95% confidence interval; CI, 1.590-4.158), p = 0.001, AA odds ratio, 1.185 (95% CI, 0.727-1.933), p = 0.496, CC odds ratio, 2.170 (95% CI, 1.515-3.106), p = 0.002

| Genotype | Odds ratio | 95% confidence interval | P value |
|----------|------------|-------------------------|---------|
| MTHFR (1298) |            |                         |         |
| AC       | 2.571      | 1.590-4.158             | 0.001***|
| CC       | 2.170      | 1.515-3.106             | 0.002***|
| AA       | 1.185      | 0.727-1.933             | 0.496   |
| Genotype | MTHFR (677) |                         |         |
| CC       | 1.2        | 0.829-1.737             | 0.334   |
| CT       | 1.6        | 1.019-2.513             | 0.041   |
| TT       | 1.333      | 0.814-2.185             | 0.253   |
for supporting this research. Finally, we thank the head and physicians of the Special Medical Centre, Tehran, Iran, for help during this research.

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