

ROLE OF POLYMORPHISM OF METHYLTETRAHYDROFOLATE-HOMOCYSTEINE METHYLTRANSFERASE (MTR) A2756G AND BREAST CANCER RISK

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Breast cancer (BC) is one of the most common causes of death among women, and second in Iran. The objectives of this study were to determine the frequency of methyltetrahydrofolate-homocysteine methyltransferase (MTR) 2756 gene polymorphism in patients with breast cancer. For the first time, we evaluated these polymorphisms and effects on the breast cancer risk association in an Iranian sporadic population-based case-control study of 282 breast cancer cases and 310 controls using a PCR-RFLP-based assay. Analyses of affected and controls show that homozygote genotype MTR 2756 AA has the highest frequency in both groups (33.3 in patients). Genotype MTR 2756 GG was the highest risk factor in our population [AG/GG odds ratio, 0.329 (95% CI: 0.146-0.741) $p = 0.006$, AA/AG, OR, 2.316, 95% CI: 1.509-3.555, $p = 0.001$, AA/GG odds ratio, 0.761 (95% CI: 0.363-1.595) $p = 0.297$]. There was a significant association of breast cancer risk with MTR 2756 GG and AA polymorphism.

Key words: *MTR A2756G* gene, polymorphism, breast cancer, *HaeIII*, PCR-RFLP.

Introduction

Little is known about the role of polymorphisms associated with breast cancer risk so not only is it studied for the first time upon our population about breast cancer but also there are different results compared to in another country.

DNA methylation is critical for regulating gene expression [1]. Methionine synthase is a vitamin B₁₂-dependent enzyme, which catalyzes the remethylation of homocysteine to methionine and the concurrent demethylation of 5-methyltetrahydrofolate to tetrahydrofolate.

Folate metabolism has an important role in carcinogenesis because of involvement in both DNA methylation and nucleotide synthesis. DNA methylation is the transfer of methyl groups (CH₃) to the C5 position of cytosine residues located in cytosine-guanine dinucleotides, by DNA methyltransferases [2] maintaining genomic and chromatin structure stability

[3, 4] and has roles including control of gene expression [5].

Methionine synthase has a role maintaining adequate intracellular folate, methionine. Methionine is an essential amino acid and is involved in methylation reactions including DNA methylation [6]. The 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*) gene is located on chromosome 1q43 [7].

A common polymorphism in the methionine synthase (*MTR*) gene (2756A → G, rs1805087) associated with lower enzyme activity than *MTR* 2756AA genotype, causing homocysteine elevation and DNA hypomethylation [8], encodes the methionine synthase enzyme (which has a role in folate metabolism), remethylating homocysteine (Hcy) to methionine [9].

There are a large number of molecular epidemiological studies on the role of *MTR* polymorphism in different kinds of neoplasm.

However, the association between polymorphism and cancer risk is still controversial. Some studies have shown

associations with colon [10], lung [11] and breast cancer [12].

Breast cancer is an increasingly important cause of illness and death among women so the aim of the present study was to attempt to elucidate a role for MTR 2756 (rs1805087) as a high risk of breast cancer using the PCR-RFLP method.

Polymorphisms in genes encoding enzymes of folate metabolism are a focus of breast cancer risk studies due to the role of these enzymes in DNA methylation, synthesis, and repair.

MTR catalyzes the remethylation of homocysteine to methionine, which is essential for maintaining adequate intracellular methionine and normal homocysteine concentrations [13].

To our knowledge, this is the first study to investigate the association between polymorphisms of the MTR gene and risk of breast cancer in the Iranian population.

We report here the allele of MTR 2756 GG association in breast cancer patients in Iran.

Material and methods

Patients' data

Analyses were conducted for 282 patients and 310 controls genotyped for MTR 2756 and ages were 35-55 years.

This study ethically was approved by the local Ethical Committee of Islamic Azad University from the point of view of patients' and also control group members' rights.

All patients participated in the Special Medical Centre, part of chemotherapy, Tehran, Iran. A questionnaire including questions on breast cancer risk factors was completed and each patient filled in a consent form. Data on the histologic type of breast cancer were phase II and III of carcinoma, and patients had metastases to lymph nodes but spread to distant organs only in a few patients in muscle, hands and behind the neck.

The blood samples were collected from patients and controls prior to the start of treatment. Subjects were

Table I. MTR 2756 genotype frequencies [n (%)] for cases and controls: Analyses of 282 affected women and 310 controls for AA MTR 2756 genotype frequency show the highest frequency in both groups (33.3), and then in A/G MTR 2756 genotype (12.3) in the case group. The AG heterozygote genotype MTR 2756 is higher in the case group (12.3) compared with controls (6.8)

STUDY GROUP	N	GG	AA	AG
Patient	282	14 (2.0%)	197 (33.3%)	72 (12.3%)
Control	310	20 (3.4%)	250 (42.2%)	40 (6.8%)
Total	609	32 (5.4%)	447 (75.5%)	113 (19.1%)

genotyped for the MTR 2756 SNP using genomic DNA extracted from peripheral blood lymphocytes. DNA was isolated from peripheral blood using the FlexiGene DNA extraction kit (Qiagen Germany).

Genotyping

The polymorphisms were detected using a modified PCR-RFLP method [14, 15]. The PCR primers were synthesized by TAG Copenhagen A/S. Primers were for each polymorphism as follows. The primers of MTR A2756G were forward 5'-TGTTCCAGACAGTTA-GATGAAAATC-3' and reverse 5'-GATCCAAAGC-CTTTTACTACTCCTC-3' [16]. The cycling conditions were 94°C, 30 s; 57°C, 30 s; 72°C, 60 s (35 cycle). The PCR products were digested with 1 unit of *HaeIII* (New England Biolabs, Beverly, MA), and the amplified fragment of 211 bp was cut into fragments of 131 and 80 bp and separated on a 6% acrylamide gel.

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 MTR polymorphisms in patient and control groups were analyzed by χ^2 and Fisher's exact tests.

Results

The aim of the present study was to attempt to elucidate a role for MTR 2756 as a high risk of breast cancer using the PCR-RFLP method, because the study was done for the first time upon our population and there have been few studies in other countries.

There was a significant association between MTR 2756 polymorphism and breast cancer risk.

Analyses of affected and controls show that heterozygote genotype MTR 2756 AA has the highest frequency in both genotypes (33.3) in the patient group.

On the other hand, even the heterozygote genotype in MTR 2756 AG has an increase in frequency compared with the control group (12.3 in patients and 6.8 in the control group) (Table I) (Fig. 1).

The P value of our results showed that the genotype MTR 2756 GG was the highest risk factor in our population: AG/GG, OR = 0.329, 95% CI: 0.146-0.741, $p = 0.006$; AA/AG, OR = 2.316, 95% CI: 1.509-3.555, $p = 0.001$; AA/GG, OR = 0.761, 95% CI: 0.363-1.595, $p = 0.297$ (Table II).

In our study there was a significant association between MTR 2756 polymorphism and breast cancer risk. So, we conclude that there is a relation between presence of MTR 2756 GG and increase of breast cancer.

Discussion

In recent years, interest in genetic susceptibility to cancers has led to growing attention to the study of polymorphisms of genes involved in tumourigenesis [17-21].

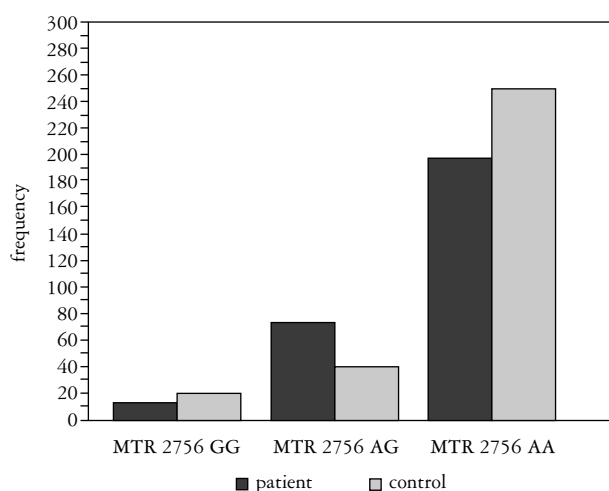


Fig. 1. MTR 2756 genotype frequencies [n (%)] (Y axes) for cases and controls (X axes): Analyses of 282 affected women and 310 controls for A/G genotype frequency show the highest frequency in both groups of patients (33.3 and 41.4) but in G/C genotype it is significant (12.6 and 6.9) in cases and the control group respectively

Functional polymorphisms in genes encoding one-carbon metabolism enzymes, methylenetetrahydrofolate reductase (MTHFR C677T), methionine synthase (MTR A2756G), methionine synthase reductase (MTRR A66G) and thymidylate synthase (TS), influence folate metabolism, but epidemiological studies have yielded inconsistent findings. In fact, they are enzymes that play a central role in the methyl group metabolic pathway, and that are involved in both DNA methylation and DNA synthesis. Two common functional polymorphisms in the *MTHFR* gene, C677T (rs1801133) and A1298C (rs1801131), have been published by us already [17]. So we decided to work upon methionine synthase (MTR A2756G).

De Cássia Carvalho Barbosa *et al.*, who worked on patients age 50 years or over, in 2012 [22], Weiner *et al.*, 2012 by a meta-analysis [23], Suzuki *et al.*, 2008 [24], and Ma *et al.*, 2009, among postmenopausal women [25], did not find any association of MTR A2756G gene polymorphisms with breast cancer risk.

Also in many studies on other cancers, scientists cannot find a significant association, for example, Jackson *et al.*, 2012 [26], in men 40-80 years old in a Jamaican population with prostate cancer and only serum folate was measured by an immunoassay method, Weiner *et al.*, 2012 [27], with prostatic cancer in the Western Siberian Region of Russia, and Daijun Zhou *et al.*, 2012, by meta-analysis [28], upon colorectal cancer (CRC) in Caucasians.

Many findings suggest that MTR function may induce breast cancer. Carvalho Barbosa *et al.*, 2012, for women ≤ 50 years, observed a risk in the presence of the polymorphic allele MTR 2756 (AG/GG, $p = 0.0118$) and, for over 50, a risk was observed with the MTHFR 677CT genotype [29], so an association

Table II. Comparison between genotypes, odds ratio and p value showed that P

GENOTYPE	OR	95% CI	P VALUE
MTR 2756			
AA/AG	2.316	1.509-3.555	0.001***
AG/GG	0.329	0.146-0.741	0.006**
AA/GG	0.761	0.363-1.595	0.297

*** $P \leq 0.001$, ** $P \leq 0.05$

with breast cancer risk was found for Brazilian women carrying the MTR A2756G polymorphic allele (AG, $p = 0.0036$; AG/GG, $p = 0.0040$). Naushad *et al.*, 2012 (plasma folate and homocysteine were measured using the Axsym folate kit and reverse phase HPLC, respectively) [30], found an association of (MTR) A2756G (OR: 4.71, 95% CI: 1.66-13.31) with breast cancer in India, Lu *et al.*, 2010, stratifying by the menopausal status, by meta-analysis [31], suggest that the MTR A2756G polymorphism may contribute to susceptibility to breast cancer among Europeans. Beetstra *et al.*, in 2008 worked upon 3 study groups (mean age 56.4 ± 2.44 years, 47.0 ± 3.12 years, and 51.0 ± 2.37 years for controls) and measured plasma folate (12.4 ± 0.9 nmol/l), plasma vitamin B₁₂ (pmol/l) was 249 ± 17 , and plasma homocysteine ($\mu\text{mol/l}$) was 7.9 ± 0.3 [32], and found that MTR A2756G was associated with increased breast cancer risk [OR: 3.2 ($p = 0.16$; 95% CI: 0.76-13.9)].

There are other studies showing that polymorphisms are present in different cancers. For example, Galbatti *et al.*, 2012 [33], and 2010 in patients at a mean age of 52.5 ± 13.7 years [34], conclude that polymorphisms are involved in the risk of head and neck cancer. Guimarães *et al.*, 2011, who worked upon patients aged under 50 years [35], and de Vogel *et al.*, 2009, in patients aged 55 to 69 [36], found an increased risk of sporadic colorectal adenocarcinoma (SCA) in a southeastern population of Brazil and the Netherlands respectively.

Uchida *et al.*, 2011, in adults aged 40-84 years [37], discovered a relation in MTR A2756G homozygotes in human age-related hearing impairment (ARHI) and in an elderly Japanese population. De Lima *et al.*, 2010 [38], suggest an association between the MTR A2756G polymorphism and retinoblastoma susceptibility in a north-east population of Brazil; Ouerhani *et al.*, 2009 [39], found the strongest result obtained with MTR 2756 in affecting bladder cancer risk; Suzuki *et al.*, 2008, in patients aged 20 to 79 years [40], suggest that the folate-related enzyme polymorphism modifies the association between pancreatic cancer risk, and Lima *et al.*, 2008 [41], suggest a role of the MTR A2756G polymorphism in multiple myeloma risk in Brazil.

In summary, we found that MTR AG was strongly associated with breast cancer risk. It may be that,

in our statistical analysis, there was a relationship between MTR AA and GG genotype and breast cancer risk, but the number of patients compared to the control group showed a decrease.

In fact, even the AG heterozygous patient number compared to the control number shows an increase.

So, in our study, we conclude that there is a relation between presence of MTR GG and increase of breast cancer risk.

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