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MTRR polymorphism and the risk for colorectal and breast cancer in Romanian patients - a preliminary study

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Rezumat

Polimorfismul MTRR și riscul de cancer colorectal și de sân la pacienții din România - studiu preliminar

Introducere: Riscul pentru cancerul colorectal (CRC) şi de sân (BC) este influențat de polimorfismele localizate în genele care codifică enzimele din calea folaților. Scopul acestui studiu a fost de a evalua dacă polimorfismul *A66G MTRR* (rs1801394) este implicat în predispoziția pentru carcinogeneza colorectală şi de sân la pacienții din România.
Materiale şi metode: În acest studiu caz-control, 300 de indivizi împărțiți în patru grupuri: pacienți cu CRC sporadic (n=120), control CRC (n=60), pacienți cu BC (n=60) şi control BC (n=60), au fost genotipați prin metoda PCR-RFLP.
Rezultate: Frecvența genotipului AA a fost de 11.7% în lotul control CRC şi respectiv de 5% în lotul BC control. Pentru loturi de pacienți, frecvența genotipului AA a fost de 9.2% pentru CRC şi de respectiv de 0% pentru BC.
Concluzii: Rezultatele obținute nu au demonstrat asocierea dintre polimorfismul *A66G MTRR* şi CRC sau BC la pacienții din România.

Cuvinte cheie: polimorfismul *MTRR*, cancer colorectal, cancer de sân, PCR-RFLP

Abstract
Background: The risk of colorectal cancer (CRC) and breast cancer (BC) is influenced by polymorphisms located in the genes encoding enzymes of the folate pathway. The aim of this study was to evaluate if *A66G MTRR* (rs1801394) polymorphism is involved in predisposition for colorectal and breast carcinogenesis in Romanian patients.
Materials and Methods: In the present case-control study, 300 individuals divide in four groups: sporadic CRC patients (n=120), control CRC (n=60), BC patients (n=60) and control BC (n=60), were genotyped by PCR-RFLP method.
Results: Frequency of genotype AA was 11.7% in CRC control and 5% respectively in BC control. For cancer groups the frequency of genotype AA was 9.2% in CRC and 0% in BC.
Conclusions: Study results do not demonstrate an association between *A66G MTRR* polymorphism and CRC or BC in Romanian patients.

Key words: *MTRR* polymorphism, colorectal cancer, breast cancer, PCR-RFLP

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Introduction

Management of patients with breast and colon carcinoma has undergone significant changes over the past two decades. The medical society aims to understand and recognize how impor-

tant the role of the pathology and biology of those cancers are in influencing the diagnosis, establishing the prognosis and selecting the therapeutic model and follow-up protocols. Screening and educational programs increased the number of cases with early stage disease and such cases need a change in the therapeutic approach. Over 50% of these patients may not need systemic adjuvant treatment but they require a strong and dense postoperative surveillance. The evaluation for the risk of appearance and the recurrence of the malignant breast or colon disease can be estimated by establishing the genetic alteration.

Impaired DNA repair synthesis and disruption of DNA methylation determined by folate deficiency may increase the risk of cancer (1). Epidemiological studies have suggested implication of low folate intake for CRC and BC risk, particularly among patients who regularly consume alcohol (2, 3).

The methionine synthase reductase (*MTRR*) enzyme restores methionine synthase (MTR) enzyme activity and plays an essential role in the folate and vitamin B12-dependent remethylation of homocysteine to methionine. Under conditions of adequate methionine, approximately 40% of homocysteine is remethylated to methionine through the activity of these enzymes (4). Thus, DNA methylation, synthesis and repair may be influenced by alterations in the function of these enzymes.

A common polymorphism *A66G MTRR* determines an amino acid substitution from methionine to isoleucine at codon 22 (M22I) (5). The *A66G MTRR* variant has a 3- to 4-fold lower affinity for MTR (6), but reports of relations with homocysteine levels are inconsistently (7, 8, 9, 10).

Few studies have investigated the association between the *MTRR A66G* polymorphism and risk of cancers. This polymorphism has been associated with a reduced risk for CRC (11, 12) and acute lymphoblastic leukemia (13), and an increased risk for malignant lymphoma (14). Meanwhile, it has not been associated with cancer risk for non-Hodgkin's lymphoma (15), uterine cancer (16) and BC (17).

The goal of this study was to assess the possible association between *MTRR A66G* (rs1801394) and susceptibility to CRC or BC in Romanian patients.

Materials and Methods

Subjects

Between January 2007 and June 2009, blood samples were obtained from 300 individuals divided in four groups: 120 sporadic CRC patients (M:W = 54:66), 60 control CRC (M:W = 27:33), 60 BC patients and 60 control BC. Medical information's regarding cancer type, tumour location and clinical evolution for patients diagnosed with CRC or BC were obtained at Cantacuzino Hospital (Bucharest) and Colțea Hospital (Bucharest). One hundred and twenty healthy controls, without known family history of malignancies and cardio-vascular diseases were selected from persons who attended N. Paulescu Institute (Bucharest) for routine analysis. The Research Ethics Committee of N. Paulescu Institute approved this study and the research is in concordance with principles of the Declaration of Helsinki. After informed consent was obtained from each participant, three ml of blood were collected in a tube containing EDTA.

Genotyping

The *MTRR A66G* polymorphism was detected by PCR-RFLP as described elsewhere (18). Briefly, about 60 ng DNA were amplified in a final volume of 10 μL, containing 1×PCR buffer, 1.5 mmol/L $MgCl_2$, 1 unit Taq DNA polymerase, 100 μmol/L dNTP, and 0.5 μmol/L of each primer (sense 5'-GCA AAG GCC ATC GCA GAA GAC AT-3' and antisense 5'-GTG AAG ATC TGC AGA AAA TCC ATG TA-3'). PCR was performed in a Corbett research thermocycler and the program consisted in an initial melting step of 1 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, and a final elongation step of 2 min at 72°C. The forward primer contained a mismatch (underlined base C in the primer sequence), which generated an NdeI restriction site when the polymorphic allele was present. The expected PCR product of 66 bp is digested into fragments of 44 and 22 bp by NdeI (Fermentas) in presence of the A allele, but remains uncut in the presence of the G allele. Products of restriction were electrophoresed (PAGE 8%) and were visualized using Bio-Imaging System after ethidium bromide staining.

Statistical analysis

The distribution of genotypes in patients and control lots was first tested for the Hardy-Weinberg equilibrium condition. The Chi-square test (χ^2, with a value of $p<0.05$ considered statistically significant) was used to compare the distribution of genotypes and alleles in patients and control groups. Because the number of AA homozygous genotype was absent in BC group, the Yates correction was applied. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by 2 x 2 contingency table using SISA programs (19). Also, Cochrane-Armitage test for trend was performed using the DeFinetti program (20).

Results

The *A66G MTRR* polymorphism was genotyped in 120 patients with CRC, 60 patients with BC and 120 healthy controls. For CRC, the male proportion was 45% both in cases and in controls. In the BC groups were only women. Mean age was for CRC group 63.7±4.8, in CRC controls 62.3±3.8, in BC group 59±3.7 and in BC controls 61.2±4.2. The colorectal tumours were localized in colon and sigmoid (62.3% of cases) and in rectum (37.7% of cases). The breast tumours were invasive ductal carcinomas in 95% of cases.

The frequencies of genotypes and alleles of analyzed polymorphism are shown in *Table 1*. The genotypes were distributed in accordance to Hardy-Weinberg equilibrium expectation for all groups, except the BC control ($p<0.05$). No statistically significant differences in the distribution of polymorphism between patients and controls have been identified.

Table 1. *The distribution of MTRR A66G genotypes and alleles between cancer and control group*

Distribution / Cancer	Genotype / alleles	Cancer N (%)	Control N(%)	OR (95%CI)[a]	χ^2(p)[b]
CRC	GG	45 (37.5)	18 (30)	1.40 (0.72-2.72)	0.98 (0.32)
	GA	64 (53.3)	35 (58.3)	0.81 (0.43-1.52)	0.40 (0.52)
	AA	11 (9.2)	7 (11.7)	0.76 (0.28-2.08)	0.27 (0.59)
	G	154 (64.2)	71 (59.2)	1.23 (0.78-1.93)	0.85 (0.35)
	A	86 (35.8)	49 (40.8)	0.80 (0.51-1.26)	0.85 (0.35)
BC	GG	23 (38.3)	25 (41.7)	0.87 (0.41-1.80)	0.14 (0.70)
	GA	37 (61.7)	32 (53.3)	1.40 (0.68-2.91)	0.85 (0.35)
	AA	0 (0)	3 (5)	-	1.36 (0.24)*
	G	83 (69.2)	82 (68.3)	1.03 (0.60-1.79)	0.02 (0.89)
	A	37 (30.8)	38 (31.7)	0.96 (0.55-1.66)	0.02 (0.89)

*Observation: [a] odds ratio and 95% confidence interval; [b] Values of Chi squares and p; *value calculated with Yate's corection*

Taking into account the absence for BC control of the subjects with AA genotype, the Yates correction was applied and the results do not accomplish the significance statistic level (p=0.24).

When we applied the Cochrane-Armitage test, there was no significant association trend between alleles and CRC (corrected OR_G = 0.78 and OR_A = 1.28; p= 0.31) or BC (corrected OR_G = 1.01 and OR_A = 1.67; p= 0.86).

Discussions

According to our knowledge, this is the first research which investigates the association between A66G *MTRR* polymorphism and CRC or BC in Romanian patients. The results show no statistically significant association between the risk for CRC or BC and analyzed polymorphism.

For control groups the frequency of genotype AA was 11.7% in CRC control and 5% in BC control, this being the first report regarding this polymorphism in Romanian population. Comparing our results with those reported by other populations, for CRC control we notice that the MAF's distribution is in interval range reported for other populations (about 39.2 – 44.5%) (21, 22). For BC control, we notice that the MAF's distribution is the smallest for our population compared with those reported (about 43.6 – 75.8%) (17, 23, 24, 25).

We observed no differences in distribution of *A66G MTRR* genotypes related to gender of patients in CRC lots (AA genotype was 7.4% in men and 15.1% in women). The difference observed for women for frequency of AA genotype between CRC and BC control (15.1% vs. 5%) may be determined by gender distribution in control lots or by selection criteria of the investigated populations.

We found that there was no statistically significant association between the risk for CRC and BC and *MTRR* A66G polymorphism. These results are in concordance with some reports about this polymorphism and CRC (22) and BC (17, 23, 24). However, for CRC an increased risk was observed for GG genotype among Japanese (26) and Caucasian subjects (27). For BC, subgroup analysis according to menopausal status show that *A66G MTRR* polymorphisms were associated with a slightly increased risk of BC in premenopausal women and may modify postmenopausal breast risk by folate consumption (25).

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