THYMIDYLATE SYNTHASE (TS) TANDEM REPEAT PROMOTER POLYMORPHISM AND SUSCEPTIBILITY TO COLORECTAL CANCER OF ROMANIAN SUBJECTS

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Abstract. The risk of colorectal cancer (CRC) is influence by polymorphisms located in the genes encoding enzymes of the folate pathway. The aim of this study was to evaluate if 2R/3R *TS* (rs34743033) polymorphism is involved in predisposition for colorectal in Romanian subjects. In the present case-control study, 75 sporadic CRC subjects and 60 healthy controls were genotyped by PCR method. The frequency of 3R/3R genotype was 40% in control group and 42.7% in cancer group. We found that there was no statistically significant association between the risk for CRC and *2R/3R TS* polymorphism in Romanian subjects.

Keywords: colorectal cancer, folate pathway, TS polymorphism, PCR-RFLP.

INTRODUCTION

Folate deficiency may increase the risk of colorectal cancer (CRC) through impaired DNA repair synthesis and disruption of DNA methylation [3]. Epidemiological studies have suggested importance of folate for CRC risk, particularly among individuals who consume alcohol [17]. The risk may be modified by polymorphisms in folate metabolizing genes [1, 10, 16].

Thymidylate synthase (*TS*) competes with *MTHFR* for the 5-methyltetra-hydrofolate as the substrate for intracellular conversion of dUMP to dTMP that represent a rate-limiting step in DNA synthesis [15].

A tandem repeat polymorphism was reported in the promoter of *TS* gene containing either two (*TS*2R*) or three (*TS*3R*) repeats of a 28-bp sequence. This polymorphism has been shown to influence gene expression [5]. Thus, individuals homozygous for triple repeats (*TS 3R/3R*) have 3.6 times higher *TS* mRNA levels compared with those homozygous for the double repeat (*TS 2R/2R*) genotype [14].

Repeated sequences in 5'-terminal domain of the *TS* are believed to regulate gene expression by forming secondary structures [8]. Overexpression of the TS protein was linked to resistance to 5-FU based treatment and associated with poor survival outcomes [13].

This polymorphism modified CRC risk and the survival rate after the disease and the response to 5-FU-based therapy [2, 8]. Also, the *TS* promoter polymorphism may be a risk factor of the colorectal adenomas [7, 18].

The goal of this study was to assess the possible association between 28 bp variable number of tandem repeat *TS* polymorphism (*rs34743033*) and susceptibility to CRC in Romanian subjects.

MATERIALS AND METHODS

Study subjects

Between January 2008 and June 2009, blood samples were obtained from 135 individuals. They have been considered as two groups: 75 sporadic CRC subjects and 60 controls. Medical information's regarding cancer type, tumour location and clinical evolution for subjects diagnosed with CRC were obtained. The 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

DNA was extracted from peripheral blood leukocytes using *Genomic Wizard DNA Purification Kit* (Promega, Madison, Wisconsin, USA) and the polymorphisms were detected by PCR as described elsewhere [6]. Briefly, about 60 ng DNA were amplified in a final volume of 10 μL, containing 1×PCR buffer, 1.5mmol/L $MgCl_2$, 1 unit Taq DNA polymerase (Promega, Madison, Wisconsin, USA), 100 μmol/L dNTP, and 0.5 μmol/L of each primer (sense 5'-CGT GGC TCC TGC GTT TCC-3' and antisense 5'-GAG CCG GCC ACA GGC AT-3'). PCR was performed in a *Corbett research thermocycler* (Corbett Research Pty Ltd, Sydney, Australia) 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 64°C, and 1 min at 72°C, and a final elongation step of 1 min at 72°C. Products of 210 bp (2R/2R), 238 bp (3R/3R) or both of these products (2R/3R) were electrophoresed (agarose 2%) and were visualized using *Bio-Imaging System* (UVP Inc, Upland, Canada) after ethidium bromide staining.

Statistical analysis

The distribution of genotypes in cancer 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 subjests and control groups. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by 2×2 contingency table using SISA

programs [20]. Also, Cochrane-Armitage test for trend was performed using the DeFinetti program [21].

RESULTS

The 28 bp variable number of tandem repeat *TS* polymorphism was genotyped in 75 subjects with CRC and 60 healthy controls. The male proportion was 56% in cases and 45% in controls. Mean age was for cases 63.7±4.8 and in controls 62.3±3.8. The colorectal tumours were localized in colon and sigmoid (54.7% of cases) and in rectum (45.3% 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 both groups. No statistically significant differences in the distribution of polymorphism between cancer and control lots have been identified.

Table 1. The distribution of *TS* genotypes and alleles between cancer and control groups

Genotype / alleles	Cancer N (%)	Control N(%)	OR (95%CI) [a]	χ^2(p) [b]
3R/3R	32 (42.7)	24 (40)	1.11 (0.55-2.22)	0.098 (0.75)
2R/3R	29 (38.7)	22 (36.7)	1.08 (0.54-2.19)	0.057 (0.81)
2R/2R	14 (18.6)	14 (23.3)	0.75 (0.32-1.73)	0.442 (0.50)
3R	93 (62)	70 (58.3)	1.16 (0.71-1.90)	0.375 (0.54)
2R	57 (38)	50 (41.7)	0.85 (0.52-1.40)	0.375 (0.54)

Note: [a] odds ratio and 95% confidence interval; [b] Values of Chi squares and p.

The distribution of analyzed polymorphism genotypes in relation with some characteristics of subjects is show in Table 2. No statistically significant differences in the frequencies of genotype have been identified in relation with gender, age of onset or localization of tumours (Table 2).

Table 2. The distribution of *TS* polymorphism in relation with some characteristics of CRC subjects

Gender of subjects / 2R/3R TS poymorphism	Male N (%)	Female N (%)	OR (95%CI)[a]	χ^2(p)[b]
3R/3R	18 (42.9)	14 (42.4)	1.01 (0.40-2.55)	0.001 (0.96)
2R/3R	15 (35.7)	14 (42.4)	0.75 (0.29-1.92)	0.351 (0.55)
2R/2R	9 (21.4)	5 (15.2)	1.52 (0.45-5.09)	0.48 (0.48)
Age at diagnosis / 2R/3R TS poymorphism	**Age < 65 years N (%)**	**Age ≥ 65years N (%)**	**OR (95%CI)[a]**	**χ^2(p)[b]**
3R/3R	13 (38.2)	19 (46.3)	1.51 (0.53-4.27)	0.613 (0.43)
2R/3R	16 (47.1)	13 (31.7)	1.91 (0.74-4.90)	1.84 (0.17)
2R/2R	5 (14.7)	9 (22)	0.61 (0.18-2.04)	0.64 (0.42)
Localization of tumors / 2R/3R TS poymorphism	**Colon + sigmoid N (%)**	**Rectum N (%)**	**OR (95%CI)[a]**	**χ^2(p)[b]**
3R/3R	17 (41.5)	15 (44.1)	0.42 (0.15-1.19)	2.68 (0.10)
2R/3R	18 (43.9)	11 (32.4)	1.63 (0.63-4.21)	1.045 (0.30)
2R/2R	6 (14.6)	8 (23.5)	0.55 (0.17-1.80)	0.969 (0.32)

Note: [a] odds ratio and 95% confidence interval; [b] Values of Chi squares and p.

When we applied the Cochrane-Armitage test, there was no significant association trend between alleles and CRC (corrected OR_{2R}= 0.87 and OR_{3R}= 1.14; p= 0.57) or characteristics of subjects (gender, age at diagnosis, and localization of tumors).

DISCUSSIONS

According to our knowledge, this is the first research which investigates the association between *2R/3R TS* polymorphism and CRC in Romanian

subjects. The results show no statistically significant association between the risk for CRC and some characteristics of individuals and analyzed polymorphism.

For controls group, the frequency of 3R/3R genotype was 40% and of 3R allele 58.3%. This represents the first report regarding this polymorphism in Romanian population. We notice that the frequency of 3R/3R genotype for our population is the highest compared with those reported for other Caucasian populations (about 29-38%) [2, 8, 11, 12]. This variation of TS genotype between populations may need to be taken into account for initiation of 5-FU therapy for Romanian CRC subjects.

Although other studies indicate that 2R/3R TS polymorphism is associated with the risk of CRC [5, 11], we found any relation for our lots. Chen and collaborators reported that compared to *TS 3R/3R* genotype, the multivariate-adjusted risk ratio was 0.86 (0.59 –1.25) for the *2R/3R* genotype and 0.59 (0.36–0.98) for the *2R/2R* genotype (*P* for trend 0.03) [2].

In our study, we don't find any relation between gender of subjects and the distribution of genotype or allele. This result is in contradiction with a previous report that shows gender difference in the benefit from 5-FU-based adjuvant chemotherapy among colorectal cancer individuals [4].

Recently, has been studied in relation with CRC other polymorphisms in the TS gene. A 6-bp deletion in the 3'untranslated region of the TS gene (TS 1494del6) has been identified [19], *del6* allele being associated with low *TS* mRNA stability and low TS expression in comparison with *ins6* allele [11]. In the second repeat of 3R alleles a G > C polymorphism has been shown to alter the transcriptional activation of the gene [21]. The *3G* allele has been associated with higher reporter gene activity at both DNA transcriptional and mRNA translational levels than the *3C* allele, and *3G*-containing genotypes (*2R/3G*, *3C/3G*, *3G/3G*) showed correlation with high *TS* mRNA expression [9].

We found that there was no statistically significant association between the risk for CRC and *2R/3R TS* polymorphism in Romanian subjects.

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