

GENETIC POLYMORPHISMS OF THYMIDYLATE SYNTHASE (TS) AND RISK OF SPORADIC COLORECTAL CANCER IN ROMANIAN PATIENTS

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REZUMAT

Introducere: Timidilat sintaza (TS) este o enzimă cheie în metabolismul acidului folic, care catalizează conversia dUMP la dTMP. Această transformare este esențială pentru a asigura provizia de timidină, nucleotid necesar pentru sinteza și repararea ADN. Secvențele repetitive de 28pb din regiunea promotor a TS (TSER 3rpt/3rpt) sunt asociate cu creșterea exprimării TS, iar deleția a 6pb din poziția 1494 din regiunea 3'UTR (TS del/ins- 6bp) afectează stabilitatea ARN. **Obiective:** Identificarea unei posibile asociații între cele două variații genetice din TS și cancerul colorectal sporadic (CCR) în populația din România; de a confirma ipoteza că factorii genetici asociați cu concentrație crescută a TS modulează riscul de a dezvolta CCR sporadic la pacienții din România. **Material și metode:** Au fost evaluați pentru prezența acestor variații genetice utilizând metoda PCR- RFLP, un număr de 69 pacienți (34 femei și 35 bărbați) diagnosticați după efectuarea examenului clinic și a colonoscopiei cu CCR sporadic, pacienți care au provenit din Clinica Chirurgie III Cluj. Grupul de control a fost format din 67 subiecți (36 femei și 31 bărbați) fără semne clinice de malignitate. **Rezultate:** Genotipurile TSER 2rpt/3rpt și 3rpt/3rpt au fost semnificativ asociate cu riscul pentru CCR sporadic (comparativ cu genotipul normal TSER 2rpt/2rpt OR = 1 (referință): TSER 2rpt/3rpt OR = 2,71 (1,59- 4,59), $p < 0,01$; TSER 3rpt/3rpt OR = 1,87 (1,08-3,19), $p = 0,02$). Polimorfismul TS del 6p a fost semnificativ asociat cu riscul pentru CCR sporadic (comparativ cu genotipul TS del/del OR = 1 (referință): genotipul TS del/ins OR = 4,73 (2,59-8,63), $p < 0,01$ și genotipul TS ins/ins OR = 5,18 (2,83- 9,43), $p < 0,01$). Genotipul TS del/del reprezintă un factor de protecție față de CCR sporadic chiar în prezența genotipurilor TSER 2rpt/3rpt sau TSER 3rpt/3rpt. Pacienții cu genotip TS del/del și TSER 2rpt/3rpt au avut un risc relativ de a dezvolta CCR sporadic de 0,87 (0,3- 2,46) (comparativ cu genotipurile TS del/del și TSER 2rpt/2rpt OR = 1 (referință)). Riscul de a dezvolta CCR sporadic a fost de 0,48 (0,14-1,64) în cazul pacienților cu genotip TS del/del și TSER 3rpt/3rpt (comparativ cu genotipurile TS del/del și TSER 2rpt/2rpt OR = 1 (referință)). **Concluzii:** Cea mai importantă interpretare a acestor date este faptul că secvențele repetitive din regiunea promotor a genei TS (3rpt versus 2rpt de 28bp) și polimorfismul TSdel6bp sunt implicate în apariția CCR sporadic în populația studiată din România.

ABSTRACT

Background: Thymidylate synthase (TS) is a key enzyme in the folic acid metabolism, which catalyzes the conversion of dUMP to dTMP. This conversion is essential to ensure the provision of thymine, a nucleotide used for DNA synthesis and repair. The 28-bp tandem repeats in the TS promoter enhancer region (TSER 3rpt/3rpt) is associated with increased expression and a 6bp deletion at position 1494 in the 3'UTR (TS del/ins- 6bp) may affect RNA stability. **Objectives:** Identification of a possible association between the two TS genetic variations and sporadic colorectal cancer (CRC) in a Romanian population; to confirm the hypothesis that genetic factors associated with high levels of TS and low TS transcript stability modulate the risk of developing sporadic CRC in Romanian patients. **Material and methods:** The clinical outcomes of 69 patients (34 females and 35 males) diagnosed after colonoscopy and clinical investigations with sporadic CRC, consecutively admitted to Surgical Clinic III Cluj, were evaluated for the presence of these genetic variations using PCR- RFLP methods. Sixty seven healthy subjects (36 females and 31 males) without any signs of malignancy formed the control group. **Results:** The TSER 2rpt/3rpt and 3rpt/3rpt genotypes were significantly associated with risk of sporadic CRC (compared with TSER 2rpt/2rpt wild- type genotype OR= 1 (reference): TSER 2rpt/3rpt OR = 2.71 (1.59-4.59), $p < 0.01$; TSER 3rpt/3rpt OR = 1.87 (1.08-3.19), $p = 0.02$). The TS del 6p polymorphism was associated significantly with the risk of sporadic CRC (compared with TS del/del genotype OR = 1 (reference): TS del/ins genotype OR = 4.73 (2.59-8.63), $p < 0.01$; TS ins/ins genotype OR = 5.18 (2.83-9.43), $p < 0.01$). The TS del/del genotype represents a protection against sporadic CRC even in the presence of TSER 2rpt/3rpt or TSER 3rpt/3rpt genotypes. Patients with combined TS del/del and TSER 2rpt/3rpt genotypes had a relative risk of 0.87 (0.3-2.46) to develop sporadic CRC (compared with TS del/del and TSER 2rpt/2rpt genotypes OR = 1 (reference)). The risk to develop sporadic CRC was 0.48 (0.14-1.64) in patients with both TS del/del and TSER 3rpt/3rpt genotypes (compared with TS del/del and TSER 2rpt/2rpt genotypes OR = 1 (reference)). **Conclusions:** The most consistent interpretation of our data appears to be that a repeat polymorphism in the TS promoter enhancer region (3rpt versus 2rpt of 28bp) and the TSdel6bp polymorphism are involved in the development of sporadic CRC in the studied Romanian population.

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INTRODUCTION

Colorectal cancer (CRC) is the fourth most common malignancy and the second leading cause of cancer deaths in Western countries. In the Netherlands, CRC is the second cause of death from malignant disease in women and the third cause of death in men.¹ Sporadic CRC involves interaction between environmental and genetic risk factors.² One of the

possible genes involved in carcinogenesis in sporadic CRC is the gene encoding thymidylate synthase (TS).

TS is a key enzyme in the folic acid metabolism and catalyses the conversion of dUMP to dTMP. This conversion is essential to ensure the provision of thymine, a nucleotide used for DNA synthesis and repair. TS is also a target for cytotoxic agents such as 5-fluorouracil, the most common chemotherapeutic agent used for the treatment of CRC.³ The target for TS is 5,10-methylenetetrahydrofolate (5,10-MTHF), which is the central metabolite in the folic acid metabolism and is used for DNA synthesis, methylation and repair. These processes are most important for the maintenance of the integrity of the genome.^{4,6}

Moreover, colon cells are subject to rapid turnover and thus are the site for high rates of DNA synthesis. At the same time, DNA repair is affected by the nucleotide availability.^{4,6} Folate deficiency or deficiency in the activity of folate-dependent enzyme may influence the risk for CRC.^{7,8}

Recently, two genetic variations named TSER 2 (2rpt/2rpt) and TSER 3 (3rpt/3rpt) 28bp-tandem repeats in the TS promoter enhancer region and a 6bp deletion at position 1494 in the 3'UTR (TS del 6bp) were found.⁷⁻¹² Patients with three tandem repeats (3rpt/3rpt) in the promoter region had higher TS levels, while patients with two tandem repeats had 3.6-fold decreased mRNA levels.¹³ Patients with TS del 6bp had decreased mRNA levels in colorectal tumors, too.^{9,10} The 3'UTR region is believed to influence TS transcript stability.¹⁴ There are two mechanisms in the carcinogenesis of CRC, supported by the hypothesis that high TS expression is associated with carcinogen-induced DNA lesions and a low folate status or deficiency in activity of folate-dependent enzyme could influence the risk for CRC.¹⁵⁻¹⁸ At the same time, high levels of TS are associated with a poor response to 5-FU and poor survival.¹⁹

Objectives: identification of a possible association between the two TS polymorphisms (TSER 3rpt/3rpt and TS del6bp) and sporadic colorectal cancer (CRC) in a Romanian population; to confirm the hypothesis that genetic factors associated with high levels of TS and low TS transcript stability modulate the risk of developing sporadic CRC in Romanian patients.

MATERIAL AND METHODS

Sample description

Blood samples were collected from 69 patients with sporadic CRC diagnosed after colonoscopy and clinical investigations at Surgical III Clinic Cluj over

Table 1. Characteristics of the studied groups.

Characteristics	Cases (N=69)	Controls (N=67)
Tumor location		
Right side, no (%)	13 (18.84%)	-
Left side, no (%)	27 (39.13%)	-
Transverse, no (%)	5 (7.24%)	-
Rectal, no (%)	24 (34.78%)	-
Age (years)		
< 55, no (%)	16 (23.18%)	19 (28.35%)
56- 65, no (%)	26 (37.68%)	25 (37.31%)
> 65 years, no (%)	27 (39.13%)	23 (34.32%)
Age (years) ±SD	63.31 ± 8.97	59.86 ± 11.91
Median	63	61
Range	43-83	26-78
Sex		
females, no (%)	34 (49.27%)	36 (53.73%)
males, no (%)	35 (50.72%)	31 (46.28%)
Smoking status		
Current smoker, no (%)	16 (23.18%)	16 (23.88%)
Smoker in the past, no (%)	3 (4.34%)	3 (4.47%)
Never smoker, no (%)	50 (72.46%)	48 (71.64%)
Alcohol consumption		
yes, no (%)	38 (55.07%)	42 (62.68%)
no, no (%)	31 (44.92%)	25 (37.31%)
Paraclinical investigations		
Plain radiograph, no (%)	12 (17.39%)	9 (13.43%)
Irrigoscopy, no (%)	2 (2.89%)	3 (4.47%)
Rectosigmoidoscopy, no (%)	5 (7.24%)	2 (2.98%)
Abdominal ultrasound, no (%)	62 (89.85%)	53 (79.1%)
Colonoscopy, no (%)	52 (75.36%)	50 (74.62%)
Relevant syndrome		
Rectorrhagia, no (%)	48 (69.5%)	43 (64.17%)
Anemia, no (%)	11 (15.94%)	4 (5.97%)
Transit disorders, no (%)	51 (73.91%)	46 (68.65%)
Subocclusion, no (%)	6 (8.69%)	-
Occlusion by luminal stenosis, no (%)	7 (10.14%)	-
Tenesmus, no (%)	9 (13.04%)	5 (7.46%)
Beginning		
less than 1 month	15 (21.73%)	-
less than 3 months	13 (18.84%)	-
less than 6 months	19 (27.53%)	-
more than 1 year	22 (31.88%)	-

the period of two years (2004 and 2005). Patients were distributed by sex, age, and tumor location (right colon, left colon, transverse colon and rectal cancer). Their mean age was 63.31 years (median 63, range 43–83, SD=8.97). Of the 69 patients clinically diagnosed with sporadic CRC, 35 were males with the mean age 64.65 years (median 64, range 43–83, SD=9.31) and 34 were females with the mean age 61.94 years (median 62, range 47–82, SD=10.42).

Blood samples were collected from a control group of 67 subjects without any signs of malignancy. Their mean age was 59.86 years (median 61, range 26–78, SD=11.91), 36 were females (mean age 60.25 years, median 60.5, range 43–73, SD=7.38) and 31 were males (mean age 60.38 years, median 62, range 26–78, SD=13.96).

All subjects were questioned about age, environmental risk factors for sporadic CRC (alcohol consumption, smoking status), any family history of cancer, and only persons without any such history were included (Table 1). All patients and subjects from the control group were Romanians.

The Scientific Ethical Committee of our University approved the present study, with participants giving their informed consent.

Methods

Genomic DNA was extracted from peripheral blood leukocytes using the method of Lahiry as described elsewhere.²⁰ The 5' end was amplified by polymerase chain reaction (PCR) using the upstream primer 5'-GTGGCTCCTGCGTTTCCCCC-3' and the downstream primer 5'-GCTCCGAGCCGGCCACAGGCAITGGCGCGG-3' on an Eppendorf thermocycler PCR machine (Eppendorf, Hamburg, Germany).⁹ The TSER two or three 28bp tandem repeats were detected after PCR amplification. The PCR reaction contained 25ng genomic DNA, 2.0mM MgCl₂, 200μM dNTPs, 10% DMSO, 0.15μM primers and 2 units of Taq DNA polymerase (SIGMA). Cycling conditions were denaturation for 10 minutes at 95°C, followed by 35 cycles of denaturation for 30 seconds at 94°C, primer annealing for 30 seconds at 59°C and elongation of primer for 1 minute and 30 seconds at 72°C. Finally, an elongation step for 2 minutes at 72°C was performed. The TSER 2rpt/2rpt variant allele was identified by the presence of the 215bp fragment, while the TSER 3rpt/3rpt variant allele was identified by the presence of the 243bp fragment (Figure 1).

The deletion of 6bp in the 3'UTR region (TS del6bp) was detected after PCR amplification with the forward primer 5'-CAAATCTGAGGGAGCTGAGT-3' and the reverse primer 5'-CAGATAAGTGGCAGTACAGA-

3'.¹¹ The PCR reaction contained 100ng genomic DNA, 2.0mM MgCl₂, 200μM dNTPs, 0.2μM primers and 2 units of Taq DNA polymerase (SIGMA). Cycling conditions were denaturation for 10 minutes at 95°C, followed by 35 cycles of denaturation for 30 seconds at 94°C, primer annealing for 30 seconds at 62°C and elongation of primer for 2 minutes at 72°C. Finally, an elongation step for 2 minutes at 72°C was performed. The size of the amplified fragment was 158bp. After PCR amplification, the 158bp fragment was digested with 5 units of DraI (New England Biolabs) for 3 hours at 37°C. The PCR fragment of 158bp was digested into 152bp for the TS del/del genotype and into 152bp and 88bp for the TS ins/ins genotype. (Fig. 2)

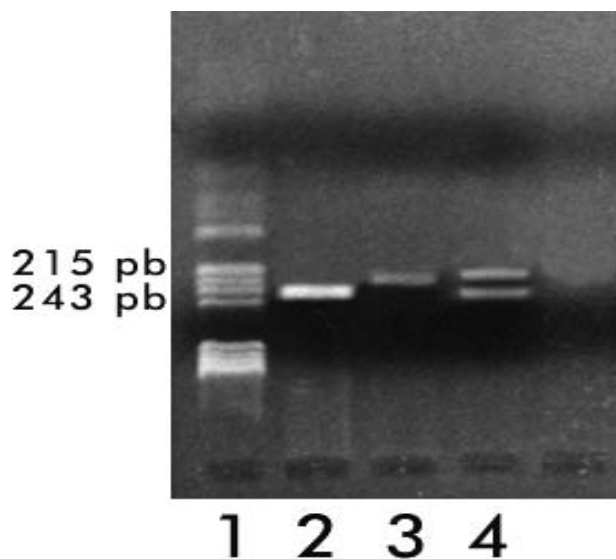


Figure 1. Agarose gel electrophoresis illustrated the amplification for TS - two and three 28bp repeats. Lane 1 - pBRHaellIDigest - DNA molecular marker; Lane 2 - TSER 3rpt/3rpt genotype: 243bp; Lane 3 - TSER 2rpt/2rpt genotype: 215bp fragment; Lane 4 - TSER 2rpt/3rpt genotype: fragments of 243 and 215bp.

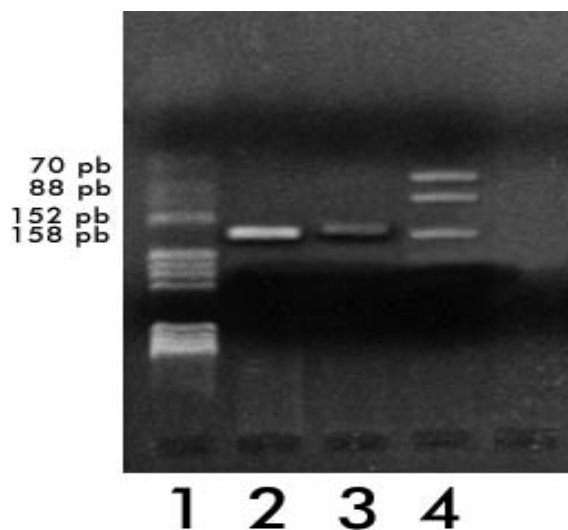


Figure 2. Agarose gel electrophoresis illustrated the amplification and enzymatic digestion of a 158bp amplified fragment. Lane 1 - pBRHaellIDigest - DNA molecular marker; Lane 2 - 158bp amplified fragment; Lane 3 - TS del/del genotype: 152bp fragment; Lane 4 - TS del/ins genotype: 152, 88 and 70bp fragments.

Statistics

Genotypic frequencies were compared with the chi square test. The relative risk to develop sporadic CRC was presented as odds ratio (OR) and 95% confidence intervals (CI) were calculated. Statistical significance was considered for a $p < 0.05$. The mean age of patients and controls was expressed as mean \pm SD.

RESULTS

The distribution of 2rpt/2rpt, 2rpt/3rpt, 3rpt/3rpt TSER genotypes in patients diagnosed with sporadic CRC versus control patients was: 24.63% vs. 19.4%, 33.33% vs. 53.73% and 42.08% vs. 26.86%, respectively. The 3rpt TSER allele occurred with a frequency of 0.58 in the group of patients with sporadic CRC compared with 0.53 in the control group. (Table 2)

For the second genetic variation, TS del 6bp, the distribution of TS del/del, del/ins and ins/ins in patients with sporadic CRC versus control patients was: 17.39% vs. 10.44%, 40.57 vs. 43.28% and 42.02% vs. 46.26% respectively. The frequency of the del6bp allele was 0.37 in the group of patients with sporadic CRC, compared with 0.32 in control subjects. (Table 2)

Table 2. Distribution of TSER (2rpt/2rpt, 2rpt/3rpt, 3rpt/3rpt) and TS (del/del, del/ins, ins/ins) genotypes in patients with sporadic CRC and controls.

Genotypes	Cases (N= 69)	Controls (N= 67)
TSER genotypes		
2rpt/2rpt, no (%)	17 (24.63%)	13 (19.4%)
2rpt/3rpt, no (%)	23 (33.33%)	36 (53.73%)
3rpt/3rpt, no (%)	29 (42.08%)	18 (26.86%)
3rpt allele, no (%)	81 (0.58)	72 (0.53)
TS del 6bp genotypes		
del/del (6/6), no (%)	12 (17.39%)	7 (10.44%)
del/ins (6/0), no (%)	28 (40.57%)	29 (43.28%)
ins/ins (0/0), no (%)	29 (42.02%)	31 (46.26%)
del6bp allele	52 (0.37)	43 (0.32)

DISCUSSIONS

Based on the results showing that TS polymorphisms are associated with the risk of colorectal adenomas, our study compared the patient group diagnosed with sporadic CRC with the control group for TS genotypes and alleles distribution.²¹

In our study we found that genotypic and allelic frequencies differed between patients with sporadic

CRC and controls.

When we compared patients with controls, we found an increased frequency of TSER 3rpt/3rpt carriers in patients compared with controls (42.08% vs. 26.86%). The TSER 2rpt/3rpt and 3rpt/3rpt genotypes were significantly associated with risk of sporadic CRC (compared with TSER 2rpt/2rpt wild-type genotype OR = 1 (reference): TSER 2rpt/3rpt OR = 2.71 (CI:1.59- 4.59), $p < 0.01$; TSER 3rpt/3rpt OR = 1.87 (CI: 1.08-3.19), $p = 0.02$). (Table 3) The results were in accordance with those obtained by Chen (2003) and Ulrich (2002).^{3,21} These results could support the hypothesis that the presence of three repeated sequences of 28bp is associated with high TS expression, associated with faster cell proliferation rates, less effective apoptosis, which can favor carcinogenesis.^{15,16}

Table 3. Risk of sporadic CRC associated with TSER and TSdel6bp polymorphisms.

Genotypes	Cases / controls	OR, 95%CI	p
TSER			
2rpt/2rpt	7/13	1.0 (reference)	
2rpt/3rpt	23/36	2.71 (1.59-4.59)	< 0.01
3rpt/3rpt	29/18	1.87 (1.08- 3.19)	0.02
TS del 6bp			
del/del (6/6)	12/7	1.0 (reference)	
del/ins (6/0)	28/29	4.73 (2.59-8.63)	< 0.01
ins/ins (0/0)	29/31	5.18 (2.83- 9.43)	< 0.01

Reference genotypes: TSER 2rpt/2rpt and TS del/del respectively; OR - odds ratio; 95%CI - 95% confidence intervals; statistically significant if $p < 0.05$

On the other hand, the distribution of TSER 2rpt/2rpt, 2rpt/3rpt and 3rpt/3rpt in control patients (19.4%, 53.73%, 26.86%) was close to that reported by Adleff (18%, 57%, 25%) and Marsh (18%, 54%, 28%).^{22,23} Moreover, the TSER 2rpt/2rpt, 2rpt/3rpt, 3rpt/3rpt genotype frequencies in our patients diagnosed with sporadic CRC (24.63%, 33.33%, 42.08%) correspond to those of Villafranca (2001) (20%, 38.5%, 41.5%) in Caucasian patients diagnosed with rectal cancer.²⁴

The TS del 6p polymorphism was significantly associated with the risk of sporadic CRC (compared with TS del/del genotype OR = 1 (reference): TS del/ins genotype OR = 4.73 (CI: 2.59-8.63), $p < 0.01$; TS ins/ins genotype OR = 5.18 (CI: 2.83-9.43), $p < 0.01$). (Table 3)

In our study, we also attempted to analyze the effect of the simultaneous presence of both TS ins/ins and TSER 3rpt/3rpt genotypes on the risk of developing

Table 4. TS combine genotypes and the risk for sporadic CRC.

TS del6bp genotypes	TSER genotypes								
	2rpt/2rpt			2rpt/3rpt			3rpt/3rpt		
	Cases/controls	OR, 95%CI	p	Cases/controls	OR, 95%CI	p	Cases/controls	OR, 95%CI	p
ins/ins (0/0)	6/5	1.53 (0.59-3.93)	0.37	7/11	2.44 (1.02-5.82)	0.03	16/15	4.72 (2.08-10.71)	< 0.01
del/ins (6/0)	7/3	1.27 (0.48-3.32)	0.6	12/22	5.33 (2.36-12.02)	< 0.01	9/4	1.69 (0.67-4.22)	0.2
del/del (6/6)	4/4	4	1.0	4/3	0.87 (0.3-2.46)	0.7	4/0	0.48 (0.14-1.64)	0.2

Reference genotype: TS del/del and TSER 2rpt/2rpt; OR - odds ratio; 95%CI - 95% confidence intervals; statistically significant if $p < 0.05$

Table 5. Distribution of TSER (2rpt/2rpt, 2rpt/3rpt, 3rpt/3rpt) and TS del 6bp (del/del, del/ins, ins/ins) genotypes by sex and age.

	TS genotypes					
	2rpt/2rpt	2rpt/3rpt	3rpt/3rpt	del/del	del/ins	ins/ins
Patients (N= 69)						
< 55 years	5 (7.24%)	6 (8.69%)	5 (7.24%)	4 (5.79%)	8 (11.59%)	4 (5.79%)
56-65 years	9 (13.04%)	8 (11.6%)	9 (13.04%)	4 (5.79%)	10 (14.49%)	11 (15.94%)
> 65 years	3 (4.34%)	9 (13.04%)	15 (21.73%)	4 (5.79%)	10 (14.49%)	14 (20.28%)
females	10 (29.41%)	7 (20.58%)	17 (50%)	10 (29.41%)	10 (29.41%)	14 (41.17%)
males	7 (20%)	16 (45.71%)	12 (34.28%)	2 (5.71%)	18 (51.42%)	15 (42.85%)
Controls (N=67)						
< 55 years	3 (4.47%)	11 (16.41%)	5 (7.46%)	2 (2.98%)	9 (13.43%)	8 (11.94%)
56-65 years	6 (8.95%)	13 (19.4%)	6 (8.95%)	4 (5.97%)	12 (17.91%)	10 (14.92%)
> 65 years	4 (5.97%)	12 (17.91%)	7 (10.44%)	1 (1.49%)	8 (11.94%)	14 (20.89%)
females	9 (25%)	20 (55.55%)	7 (19.44%)	5 (13.88%)	13 (36.11%)	18 (50%)
males	4 (12.9%)	16 (51.61%)	11 (35.48%)	2 (6.45%)	16 (51.61%)	13 (41.93%)

sporadic CRC. Patients with both TS del/ins and TSER 2rpt/2rpt had a 1.27 higher (CI: 0.48-3.32) increased risk of sporadic CRC (compared with TS del/del and TSER 2rpt/2rpt genotypes OR = 1 (reference)). (Table 4) The risk of sporadic CRC increased to 1.53 (CI: 0.59-3.93) in patients with both TS ins/ins and TSER 2rpt/2rpt genotypes (compared with TS del/del and TSER 2rpt/2rpt genotype OR = 1 (reference)). Patients with both TS ins/ins and TSER 2rpt/3rpt genotypes had a 2.44-fold (CI: 1.02-5.82) increased risk of sporadic CRC (compared with TS del/del and TSER 2rpt/2rpt genotype OR = 1 (reference)). The risk of sporadic CRC increased to 4.72 (CI: 2.08-10.71) in patients with both TS ins/ins and TSER 3rpt/3rpt genotypes (compared with TS del/del and TSER 2rpt/

2rpt genotype OR = 1 (reference). Very interestingly, the presence of TS del/del genotype represents a protection against sporadic CRC even in the presence of TSER 2rpt/3rpt or TSER 3rpt/3rpt genotypes. Patients with combined TS del/del and TSER 2rpt/3rpt genotypes had a relative risk of 0.87 (0.3-2.46) to develop sporadic CRC (compared with TS del/del and TSER 2rpt/2rpt genotypes OR = 1 (reference)). The risk to develop sporadic CRC was 0.48 (CI: 0.14-1.64) in patients with both TS del/del and TSER 3rpt/3rpt genotypes (compared with TS del/del and TSER 2rpt/2rpt genotypes OR = 1 (reference)). (Table 4)

When the distribution of these two genetic variations in patients with sporadic CRC was analyzed depending on age and by sex, we obtained

the following results: the frequency of the TSER 3rpt/3rpt genotype was higher in patients aged over 65 years (21.73%) compared to 7.24% and 13.04% in patients aged less than 55 years and patients aged between 56-65 years, respectively. The TSER 3rpt/3rpt genotype was more frequent in females with sporadic CRC (50%) compared to males with sporadic CRC (34.28%). The frequency of the TSER 2rpt/3rpt genotype was also higher in patients over 65 years old (13.04%) compared to 8.69% and 11.6% in patients less than 55 years old and patients between 56-65 years old, respectively. The TSER 2rpt/3rpt genotype had a different sex distribution compared with the TSER 3rpt/3rpt genotype. The TSER 2rpt/3rpt genotype was more frequent in males with sporadic CRC (45.71%) compared to females with sporadic CRC (20.58%).

For TS del 6bp genetic variation, we obtained the following results: the TS ins/ins genotype was more frequent in patients over 65 years old (20.28%) compared to 15.94% and 5.79% in patients aged between 56-65 years and in patients aged less than 55 years, respectively. The TS del/ins genotype had the same frequency in patients aged between 56-65 years and in patients over 65 years old, 14.49%. The frequency of this genotype was 11.59% in patients aged less than 55 years. The TS ins/ins genotype did not significantly differ in women with sporadic CRC compared with men (41.17% vs. 42.85%), while the TS del/ins genotype was more frequent in men (51.42%) than in women (29.41%).

CONCLUSIONS

The most consistent interpretation of our data appears to be that a repeat polymorphism in the TS promoter enhancer region (3rpt versus 2rpt of 28bp) and the TSdel6bp polymorphism are involved in the development of sporadic CRC in the studied Romanian population. In our study, the association between sporadic CRC and the genetic variations in the TS gene may reflect a genetic similitude between the Romanian and Caucasian populations. On the other hand, Romanian patients with sporadic CRC had higher frequency of combined TSER 3rpt/3rpt and TS ins/ins genotypes. It means that this genotype combination represents a higher risk of developing sporadic CRC.

It was demonstrated that folate modulates the effect of TS genotypes in carcinogenesis, the TSER 3rpt/3rpt decreased the risk for colorectal adenomas when associated with high folate intake, while the TSER 2rpt/2rpt had a lower risk when associated with

low folate intake.²¹ The diet of Romanian population is characterized by high protein and animal fat intake and low folate and fiber intake, which could explain the risk of sporadic CRC associated with the TSER 3rpt/3rpt genotype. However, further studies are needed in order to confirm this genetic predisposition.

The findings of this study do not exclude the possibility that other variations in another gene such as the gene encoding the methylenetetrahydrofolate reductase (MTHFR) and the gene encoding enzymes implicated in detoxification may be involved in the etiology of sporadic CRC. For instance, an association between the polymorphisms in N-acetyl-transferase 2 (NAT2), glutathione S-transferase M1 (GSTM1) and sporadic CRC has been reported.

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