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Polymorphisms (C677T and A1298C) of MTHFR Gene in Sporadic Gastro-intestinal Cancers in Tunisian Population: Interaction with Dairy Products, Treatment and Prognostic Factors

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Abbreviations: MTHFR: 5,10-Methylenetetrahydrofolate Reductase; PCR: Polymerase Chain Reaction; RFLP: Restriction Fragment Length Polymorphism; CI: Confidential Interval; OR: Oods Ratio; TNM Stage: Tumor Node Metastasis Stage; CRC: Colorectal Cancer; GC: Gastric Cancer; ADK: Adenocarcinome; Glu: Glutamine; Ala: Alanine; Val: Valine; 5-FU: 5 Fluorouracil

Introduction

Colorectal and gastric cancers are multifactor diseases. There is more than one cause of occurrence for gastrointestinal cancer. Furthermore, many environmental factors increase the risk of colorectal neoplasia [1] and gastric cancer. The interaction between gene and environment including lifestyle was involved in numerous studies. Thereby, several epidemiological studies have mentioned the interaction between folate intake and certain cancers [2,3]. Low folate level has been associated with increased risk for gastric cancer [4] and colorectal cancer [5]. Into cell, folate may be in the form of 5-methylenetetrahydrofolate which can be responsible for remethylation of the precursor of the S-adenosylmethionine (SAM). SAM is a major donor of methyl group for DNA [6] into metabolism of homocysteine and folate which involves several enzymes that regulate DNA synthesis and DNA methylation such as MTHFR enzyme. MTHFR catalyzes the formation of 5-methyl THF from methylene-THF 5.10 [7-9]. This methyl derivative THF is necessary for the remethylation of homocysteine to methionine. The MTHFR gene was localized by Goyette et al. in 1994 in the chromosome 1 [7]. The MTHFR gene includes 11 exons [8]. Later, Gaughan et al. showed that it does not contain TATA box region, but contains several CpG islands which are very important as be siding for transcription factors. The C677T (rs1801133) polymorphism (substitution of alanine for valine), makes MTHFR enzyme thermolabile with a decrease of 50% for its activity [10]. The concentration of total homocysteine is particularly high among subjects with 677TT genotype in the case of folic deficiency [11,12] or folate insufficient dietary intake [12,13]. There are other polymorphisms in MTHFR gene the most common is A1298C (rs1801131) which causes a substitution at codon 429 glutamate to alanine. MTHFR 1298A>C (Glu>Ala) polymorphism may also lead to lower activity of the enzyme [14] but polymorphism alone does not influence the concentration of Homocysteine. Hence single polymorphism by itself (SNPs) may modify folate status and cause colorectal cancer [15]. In this study we have evaluated the possible association of MTHFR C677T and MTHFR A1298C polymorphisms with risk of stomach and colorectal cancer. Indeed several studies have reported the association of MTHFR polymorphisms with cancer, thus, Polymorphisms 677C>T and 1298A>C have been evaluated in relation with breast cancer [16], endometrial cancer [17], colorectal cancer [18,19] and gastric cancer [20,21].

Few studies have reported an interaction between gene and environment as risk of CRC and gastric cancer. Moreover, alcohol intake is considered as a risk for gastrointestinal cancer with an effect on folate, besides studies have reported a positive association of MTHFR gene polymorphism and alcohol consumption with gastrointestinal cancer [22].

In this work we have undertaken the combined effect of environmental factors, genetic and clinical outcomes with occurrence of gastro-intestinal cancer in Tunisian population.

Patients and Methods

Study population

In this study, 68 gastric cancer cases and 95 colorectal cancers were recruited between 2008-2012 from institute of oncology «Salah Azaiz» in Tunisia. All cases were newly diagnosed and considered before any treatment. All patients presented with histopathology confirmed adenocarcinoma cancer (ADK).The mean age of the patients at diagnosis was 57.2 with a range from 20 to 89 years, data concerning Tumor types and stages were determined in department of pathology and regrouped only 70 colorectal cancers and 17 gastric cancers; the data collected included stage, lymph node status, differentiation and histological type of tumor, besides we collected data concerning chemotherapy and surgery treatment. Same patients received Folfox treatment as well as Folfiri combination, whereas other received monotherapy treatment such as xeloda and LV5-FU2. Patients were assessed before the initiation of chemotherapy and every 2 weeks during treatment. On the one hand 185 healthy controls were recruited from department of gastroenterology at the Charles Nicole Hospital; these controls were considered without any history of malignancy, and the mean age was 56.01 years with a range from 20 to 85 years.

Cases and controls were interviewed by a validated food survey

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which contains a Food Frequency Questionnaire (FFQ) [23] developed by the authors to determine individual habits such dairy products intake (milk, yogurt, cheese) and alcohol drinking

We collected 5 ml of peripheral blood to each subject in EDTA tube, stored at -20°C. The collection of blood samples was approved by the Ethics committee.

Genotyping of polymorphisms of MTHFR gene

To genotype MTHFR gene we have included only 137 healthy controls, 78 colorectal cancers and 43 gastric cancers. For MTHFR C677T (rs1801133) cases and controls were genotyped by PCR-RFLP method as reported by Frosst et al. [10] the primers for the 677 site were F-GCCTCTCCTGACTGTCATCC- and R-TCACAAAGCGGAAGAATGTG-. Thermocycling conditions of MTHFR C677T (rs1801133) polymorphism were: 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 56°C for 3 s, 72°C for 30 s with a final extension step of 72°C for 10 min. The restriction enzyme HinfI (New England BioLabs) was used to distinguish the C677T polymorphism. The 677CC wild-type genotype was identified by the presence of a 198 pb; the 677CT heterozygous genotype by 198, 175 and 23 pb; and 677TT mutant genotype by 175 and 23 pb. Polymorphism of MTHFR A1298C (rs1801131) in patients and subjects was genotyped by fluorescent-based restriction fragment length polymorphism method and using the following primer pairs: forward primer Fam -GCAAGTCCCCCAAGGAGG, reverse primer -GGTCCCCACTTCCAGCA- The forward primers were fluorescently labeled, The PCR amplification was performed in a total 10 µl mixture containing: 50 ng genomic DNA, 0,4 µM of each primer (each deoxynucleotide triphosphate at 400 µM), 1,5 mM $MgCl_2$ contained in 10X PCR Buffer, 0.4 U AmpliTaq DNA polymerase (Applied biosystems, Evry, France). Thermocycling conditions of MTHFR A1298C (rs1801131) polymorphism were: 94°C for 5 min followed by 35 cycles at 94°C for 1 min, 64°C for 1 min and 72°C for 1 min with a final extension step of 72°C for 10 min. Fluorescently labeled PCR products were incubated overnight at 37°C with the restriction enzyme MboII (New England BioLabs).The digested PCR product was diluted 1:10 in H_2O. A volume of 2 µl of the digested PCR product was mixed with 0.3 µl of internal size standard and 9.7 µl of deionised formamide, denatured and separated on automated sequencer ABI 3130 genetic analyzer (ABI). The two alleles were easily separated using this method: the A allele, resulted in an undigested PCR product with 3 fragments 79 bp, 37 pb, 29 pb; the C allele, resulted in a digested PCR product with two fragments of 108 and 37 bp; as the 108-bp fragment contained the fluorescently-labeled forward primer it was visualized on the ABI-3130. Allele typing was performed with Gene Mapper software (Applied biosystems, Evry, France).

Statistical analysis

The data were analyzed using SPSS software (version 11.5.). Significance of the association was determined by Pearson's chi-squared test χ^2 and Fisher's exact test. A value of $p<0.05$ was considered as significant. The measure of the association between MTHFR genotype and risk of cancer was estimated by the ORs and 95% confidence intervals.

Interaction of genotype with alcohol consumption, dairy products, and data concerning TNM staging, anatomical site, and lymph node status were estimated using the logistic regression model.

Results

The characteristics of population are listed in table 1, the age range of cases was between 20 and 89 years old, for controls it was between 20 and 85 years old the mean age was similar in cases and controls. A statistically significant sex difference was observed between gastric

Characteristics	Controls	CRC	Gastric cancer
No	185	95	68
Mean age(years)	56,01	56,44	57,96
Gender (male/female)	92/93	49/46	49/19*
Age-range	20-80	28-80	20-89
Alcohol			
No	166(91)	62(82)	32(76)*
Yes	19(9)	13(18)	10(24)
Milk consumption			
<3/week	83(45)	66(69)**	46(67)
≥3/week	102(55)	30(31)	23(35)
Cheese			
<2/week	116(63)	64(62)	46(67)
≥2/week	69(37)	32(38)	20(33)
Yogurt			
<2/week	111(69)	57(59)	34(49)
≥2/week	74(31)	39(41)	35(51)
No		74	17
TNM stage			
I		4(5.4)	-
II		11(14.9)	2(11.8)
III		36(48.6)	2(11.8)
IV		15(20.3)	1(5.9)
Not determined		8(10.8)	12(70.5)
Lymph nodes status			
4 nodes		53(48.7)	2(5.9)
>4 nodes		15(5.4)	4(11.8)
Not determined		6(8.1)	11(70.5)
Differentiation			
Poorly differentiated		1(1.4)	4(23.5)
Moderately differentiated		27(36.4)	7(41.2)
Well differentiated		43(58.1)	5(29.4)
Not determined		3(4.1)	1(5.9)
Histological type			
ADK		26(35.1)	10(58.8)
ADK Luberkhunien		28(37.8)	1(5.9)
ADK infiltrant		6(8.1)	1(5.9)
Other type of ADK		14(19)	5(29.4)
Surgery			
No		7(9.5)	5(29.4)
Palliative		31(41.9)	6(35.3)
Radical		36(48.6)	5(29.4)
Not determined		-	1(5.9)
Neo-adjuvant chemotherapy			
No		52(70.3)	12(70.6)
Yes		22(29.7)	3(17.6)
Not determined		-	2(11.8)
Adjuvant treatment			
No		19(25.7)	7(41.2)
Yes		55(74.3)	9(52.9)
Not determined		-	1(5.9)

*Gastric cancer compared with controls, p<0.05; **Colorectal cancer compared with controls, p<0.05

Table 1: General characteristics of population.

cancer and healthy controls. By comparing beverage behavior with genotypes we found that alcohol consumption is positively associated with increase risk of gastric cancer (P=0.01; OR=3.24; CI =1.19-8.37) but not with colorectal cancer. Furthermore, we found that high intake of milk more than -three times per week- is protective against colorectal cancer (P=0.03; OR=0.55; CI=0.33-0.92), besides consumption of milk more than-three times per week in CRC grouped with GC compared with controls is protective factor ($P=1.510^{-5}$, OR=0.39; CI=0.24-0.61) .Although consumption of cheese and yogurt haven't any effect on occurrence of gastro-intestinal cancer. In addition clinical characteristics and treatment are grouped.

Table 2 shows the genotype distribution of MTHFR C677T and MTHFR A1298C among cases and controls, the distribution of the MTHFR SNPs was in Hardy-Weinberg equilibrium in both groups.

Concerning MTHFR C677T polymorphism, the frequencies of wild-type, heterozygous and mutant genotypes were respectively; 56.9, 38.7 and 4.4% in controls, 55.8, 39.5 and 4.7% in gastric cancer and 41, 48.7 and 10.3% in CRC. TT genotype is associated with risk of colorectal cancer (p=0.034; OR=3.25, CI 95% (1.044-10.118)) compared with CC genotype, as well as T allele (p=0.015; 1.702, CI 95% (1.106-2.621)), meanwhile there were no significant differences in genotype distribution between cases with gastric cancer and controls. Furthermore if we compare CC+TT genotype with wild type genotype CC we do not found an association with GC group, however in CRC group we have shown a risk of CC+TT genotype compared with wild type genotype (P=0.024, OR=1.90, 95%CI=1.04-3.48).

Concerning MTHFR A1298C polymorphism, the frequencies of wild-type, heterozygous and mutant genotypes were respectively; 51.3, 43.6 and 5.1% in controls, 68.6, 25.7 and 5.7% in gastric cancer and 65.3, 29.2 and 5.6% in CRC. The distribution of MTHFR A1298C genotypes and alleles was similar in controls compared with gastro-intestinal cases; MTHFR1298 polymorphism is not associated with risk of gastric and colorectal cancer in our study, besides we have shown no association between AC+CC genotypes compared with AA genotype in GC as well as CRC.

Considering linkage disequilibrium, we established the haplotypes of MTHFR gene taking into account genotypes at both SNPs (677 and 1298). Combined genotypes of MTHFR gene were compared in a case control study (Table 3). So we have observed that combined genotype CA/CC versus CA/TA is associated with risk of colorectal and gastric cancer (p=0.02, OR=2.80, 95%CI (1.03-7.67)).

The CA/CC genotype is less frequent in gastro-intestinal group, however when we compared CA/CC genotype versus CA/TA genotype between controls and CRC+GC we found a risk against cancer (p=0.02, OR=2.80; 95%CI=1.03-7.67). Moreover CA/TA genotype compared with other genotypes appeared protective against CCR (P=0.035; OR=0.47, 95%CI=0.21-10.1).

Furthermore we analyzed the distribution of MTHFR haplotypes and their effect on gastro-intestinal cancer (Table 4 and 5). While four possible haplotypes for MTHFR were observed in cases and controls, we have observed that the most common haplotype (CA) is associated with GC (P=0.05; OR=0.46, 95%CI=0.19-1.10).

We have listed some parameters of clinical outcomes in Table 6, such as TNM stage, lymph nodes status, differentiation and histological type of cancer. We have not observed significant differences according to stages of colorectal cancer group with MTHFR C677T distribution. Furthermore, when we compared MTHFR 677 genotypes of patients

MTHFRC677T Controls, n=137 Gastric cancer, n=43 CRC, n=78				GC	CRC	
				P value	P value	OR 95%CI
CC	78(56.9%)	24(55.8%)	32(41%)			
CT	53(38.7%)	17(39.5%)	38(48.7%)	0.908	0.060	
TT	6(4.4%)	2(4.7%)	8(10.3%)	0.925	0.034	3.25(1.044-10.118)
C	209(76.3%)	65(75.6%)	102(65.4%)			
T	65(23.7%)	21(24.4%)	54(34.6%)	0.900	0.015	1.702(1.106-2.621)
MTHFRA1298C	Controls, n=78	GC, n=35	CRC, n=72			
AA	40(51.3%)	24(68.6%)	47(65.3%)			
AC	34(43.6%)	9(25.7%)	21(29.2%)	0.068	0.065	
CC	4(5.1%)	2(5.7%)	4(5.6%)	0.827	0.827	
A	114(73.1%)	57(81.4%)	115(79.9%)			
C	42(26.9%)	13(18.6%)	29(20.1%)	0.18	0.17	

Table 2: Genotypes distribution of MTHFR C677T polymorphism and MTHFR A1298C among gastrointestinal cancers and controls.

Combined genotype (MTHFR gene)	Controls, n=79	CCR, n=73	Gastric cancer, n=33	P value	OR 95%CI
CA/CA	19	17	13	0.10**	
CA/CC	18	10	4	0.08*	
CA/TA	17	27	10	0.035***	0.47 (0.21-10.1)
CA/TC or CC/TA	15	10	4		
CC/CC	4	2	1		
CC/TC	1	2	0		
TA/TA	3	3	1		
TA/TC	1	2	0		
TC/TC	1	0	0		

P* value was calculated with CA/CC versus other genotypes, between CCR+GC compared with controls
P** value was calculated with CA/CA versus other genotypes, between GC compared with controls
P*** value was calculated with CA/TA versus other genotypes, between CCR compared with controls

Table 3: MTHFR C677T and A1289C combined genotype in gastrointestinal cancer.

Haplotypes	Controls (n=79)	CCR (n=73)	P value
CA	0.51	0.52	0.28
CC	0.21	0.14	
TA	0.19	0.27	0.35
TC	0.08	0.07	

Table 4: Association between MTHFR haplotypes and colorectal cancer.

Haplotypes	Controls (n=79)	GC (n=33)	P value OR95%
CA	0.51	0.63	0.05 0.46(0.19-1.10)
CC	0.21	0.12	
TA	0.19	0.21	0.07
TC	0.08	0.03	

Table 5: Association between MTHFR haplotypes and gastric cancer.

with nodes <4 (CC, 40%, CT, 56% and TT, 4%) and patients with nodes ≥4, we did not find any differences. The same result was found for MTHFR 1298 polymorphism, no statistical differences in genotype distribution were observed between TNM stages, differentiation and histological types.

Moreover a total of 70 patients with CRC received different regimes of chemotherapy treatment (neoadjuvant and adjuvant treatment) which contained capecitabine, LV5-FU and combination treatment folfox and folfiri, the association of MTHFR polymorphisms was not significantly different between 5FU with oxaliplatin group as well as irinotecan with 5FU group and 5FU without oxaliplatin group, besides there were not significantly different between MTHFR genotype distribution and patients treated by capecitabine (Table 7).

Concerning effect of environment factors we have summarized the interactions between MTHFR polymorphisms, alcohol consumption and dairy products on Tables 8 and 9.

We have observed an additive effect of CT+TT genotype on alcohol intake and dairy product, however there is a significant difference in alcohol intake ($p=110^{-6}$; OR=6.10; 95%CI (2.43-15.55) in CRC with MTHFR C677T polymorphism if we compare CC genotype of no drinkers versus CT+TT genotype of drinkers, whereas the risk effect of alcohol on GC was eliminated by additive effect, but concerning MTHFR 1289 group, we found a protective effect of AA genotype in non drinkers compared with drinkers. Furthermore we did not observe any association in alcohol intake in GC in both polymorphisms. We have also shown that within group the consumption less than three times per week of milk is a risk for CRC if we compare CC genotype with CT+TT(p=0.002, OR=3.14; %95CI=1.37-7.26).

The consumption of cheese is also protective in MTHFR677 group (p=0.02, OR=0.31; %95CI=0.09-0.98) and in MTHFR1298 group, however concerning the consumption of Yogurt we found that within the group who consumes less than twice a week the CT+TT genotype presents a risk for CRC (p=0.0008, OR=3.94; %95CI=1.61-9.80), this effect was not found in MTHFR1298 group. Furthermore we found that there is no association between several polymorphisms and dairy product in patients with GC.

Discussion

In the present paper based on case-control studies we have investigated the possible association between MTHFR gene polymorphism and susceptibility to gastrointestinal cancer.

MTHFR is an important enzyme that plays a crucial role in methylation mechanism. Its deficiency may cause aberrant DNA methylation in the cell and DNA instability [24]. Two SNPs of MTHFR gene have been considered in this study. For the SNP at position 677, the prevalence of variant allele "T" and mutant genotype MTHFR 677 TT, varies among different ethnicity and this is in relation to interaction with gene-environment. The higher prevalence is reported in Italian and French populations, in our study we have found a high prevalence of TT genotype (10.3%) similar to that described in Kashmiri population [25]. In this study the frequency in control groups of MTHFR TT is 4.4% which is slightly lower than in other studies on the Tunisian population [26]. Many studies suggested that MTHFR polymorphism is associated with CRC and gastric cancer. The prevalence of MTHFR 677TT is variable in cases with gastric cancer. We have found for mutant TT a frequency of 4.7% which is low compared to the frequency found in patients with gastric cancer in Chinese population [27]. In a case/ control study, we did not reveal any association of MTHFRC677T with gastric cancer, some studies in China [28,29] and Denmark [30] elucidated that TT genotype at position 677 of MTHFR gene, cause an important risk of gastric cancer, though other studies did not find this association [31,32]. Moreover, several studies in Mexico [33] and Korea [19] indicated a protective role of MTHFR 677 TT in the development of gastric cancer.

Meanwhile, in our study we revealed MTHFR 677 TT genotype as risk factor for CRC (p=0.034; OR= 3.25; 95%CI (1.04-10.11)) compared with controls. Our results confirmed what was found in Romanian population [34] and recent meta-analyses in China [35]. However, other studies have found a protective effect of TT genotype against CRC [36].

Concerning MTHFR A1298C polymorphism a prevalence frequency of MTHFR 1298 CC was (5.1%) and was almost similar to that previously reported for healthy Tunisians [21]. But we did not find any association with colorectal and gastric cancer, which is consistent with study relived in Cambridge and China [18,37]. If we consider the haplotype analysis based on the two polymorphisms of MTHFR gene (C677T and A1298C) we show that CA haplotype was associated with GC (p=0.05; OR=0.46; 95%CI=0.19-1.10) compared with controls, however other haplotypes analysis for the polymorphism in MTHFR gene do not show any association with colorectal and gastric cancer. The analyses of haplotypes add information on recombination (physical exchange of DNA during meiosis) causing variants and mutations by linkage methods [38]. A combined effect of the MTHFR C677T variant genotype and A1298C variant genotype on the risk of gastric cancer and CRC was observed in previous studies [21,39]. We found that the risk of wild homozygous CA/TA variant genotype compared to other genotypes is protective against CRC (p=0.035; OR=0.47; 95%CI=0.21-10.1). However we have shown a risk of CA/CC genotype compared with CA/TA (p=0.02, OR=2.80; 95%CI=1.03-7.67).

folate is necessary for generating a new cell [40] and his implication in DNA synthesis, methylation and repair, thus same nutrients involved in folate metabolism such (Vitamin B12, methionine…) and alcohol intake have an effect on folate level on cancer risk. It has been reported in some studies that alcohol intake influences metabolism of folate by reducing folate absorption and inhibiting methylation and deactivating methionine synthase [41]. Moreover alcohol intake may modify the inverse association between folate intake and plasma homocysteine level [42].

Taking this data into account, we investigated the role of alcohol intake we have indeed shown a risk of alcohol consumption in patients with gastric cancer while frequent consumption of milk -more than

		MTHFR C677T				MTHFR A1298C		
	CC	CT	TT	P* for interaction	AA	AC	CC	P *for interaction
Colorectal cancer (n=74)								
Stage								
I	0	2(50)	2(50)	0.43	2(100)	0	0	0.53
II	3(30)	7(70)	0		5(45.5)	4(36.4)	2(18.2)	
III	14(41.2	18(52.9)	2(5.9)		20(62.5)	9(28.1)	3(9.4)	
IV	8(53.3)	6(40)	1(6.7)		8(72.7)	3(27.3)	0	
Lymph node status								
<4 nodes	10(40)	14(56)	1(4)	0.42	16(69.6)	4(17.4)	3(13)	0.45
≥4 nodes	6(54.5)	4(36.4)	1(9.1)		5(55.6)	4(44.4)	0	
Differentiation								
Moderately differentiated	12(48)	12(48)	1(4)	0.23**	11(55)	7(35)	2(10)	0.34
Well differentiated	14(33.3)	25(59.5)	3(7.1)		27(67.5)	10(25)	3(7.5)	
Histological type ADK	7(30.4)	12(52.2)	4(17.4)	0.24	12(60)	6(30)	2(10)	0.60
ADK Luberkhunien	13(46.4)	14(50)	1(3.6)		12(52.2)	9(39.1)	2(8.7)	
ADK infiltrant	3(50)	3(50)	0		6(90)	1(10)	0	
Other type of ADK	4(16.2)	8(80)	1(3.8)	0.42	10(65)	3(31.2)	1(3.8)	0.46

P*: For MTHFR C677T, CC as reference group and CT grouped with TT; for MTHFR A1298C AA as reference grouped with AC+CC
P**: For differentiation was calculated between moderately and well differentiated

Table 6: Interaction between MTHFR polymorphisms and prognostic factor with colorectal and gastric cancer.

	MTHFRC677T MTHFRA1298C							
	CC	CT	TT	P for interaction	AA	AC	CC	P for interaction
Colorectal cancer (n=70)								
Surgery								
No	0(0%)	5(83.3%)	1(16.7%)		4(100%)	0(0%)	0(0%)	
Palliative	11(36.7%)	16(53.3%)	3(10%)	0.28	17(65.4%)	7(26.9%)	2(7.7%)	0.58
Radical	16(47.1%)	16(47.1%)	2(5.9%)		19(57.6%)	11(33.3%)	3(9.1%)	
Neoadjuvant chemotherapy								
No	21(42%)	26(52%)	3(6%)	0.35*	26(59.1%)	14(31.8%)	4(9.1%)	0.26*
Yes	6(30%)	11(55%)	3(15%)		14(73.7%)	4(21.1%)	1(5.3%)	
Kind of chemotherapy								
Xeloda	5(83.3%)	7(63.6%)	3(100%)		11(78.6%)	2(50%)	1(100%)	
Folfiri	0(0%)	1(9.1%)	0(0%)	0.69	0(0%)	1(25%)	0(0%)	0.35
Folfox	1(16.7%)	3(27.3%)	0(0%)		3(21.4%)	1(25%)	0(0%)	
LV5-FU2 Adjuvant chemotherapy								
No	5(31.3%)	8(50%)	3(18.8%)	0.49*	8(61.5%)	5(38.5%)	0(0%)	
Yes	22(40.7%)	29(53.7%)	3(5.6%)		32(64%)	13(26%)	5(10%)	0.86*
Kind of chemotherapy								
Xeloda	0(0%)	2(6.9%)	0(0%)		0(0%)	2(15.4%)	1(20%)	
Folfiri	3(13.6%)	0(0%)	0(0%)	0.29	3(9.4%)	0(0%)	0(0%)	0.17
Folfox	17(77.3%)	22(75.9%)	3(100%)		25(78.1%)	8(61.5%)	4(80%)	
LV5-FU2	2(9.1%)	5(17.2%)	0(0%)		4(12.5%)	3(23.1%)	0(0%)	

P was calculated between CC versus CT+TT genotype for MTHFRC677T, For MTHFR A1298C P was calculated between AA versus AC+CC genotype

Table 7: interaction of treatment with gastrointestinal cancer.

three times per week- is protective against colorectal cancer however no effect of yogurt and cheese was shown.

Several epidemiologic studies examined the interaction between dairy product and gastro-intestinal cancer but their findings have been inconclusive. Dairy products contain also Vitamin B12, or cobalamin. Milk is an essential source of Vitamin B12 and interferes with folate metabolism.

A meta-analysis of 10 cohort studies (more than 500 000 patients followed for 6-16 years, 4,992 colorectal cancers), published in 2004 shows that the consumption of milk (and calcium) is associated with a decrease of approximately 15% risk of cancer colorectal, the analysis suggests a threshold effect at about 1000 mg/day of calcium [43].

Since then, four large cohort studies, including a French study [44-47] confirm the protective effect of dairy products against the risk of adenoma or cancer, in both men and women, with a decrease risk of 15 to 50% depending on the study. The effect is mainly due to calcium: for example, in the E3N-EPIC study (70000 French followed for 7 years) the adenoma risk is reduced by 20% in women who consume more than 424 g/d of dairy products (vs. less 185 g/d), and 14% of those who consume more than 736 mg/d of dairy calcium (vs. less than 360 mg/d).

MTHFR C677T	Controls (n=137)	CRC (n=78)	P	OR	MTHFR A1298C	Controls(n=78)	CRC(n=72)	P	OR
Drinking habit; no					Drinking habit; no				
CC*	69	20	NS		AA*	22	34	0.009	0.32(0.12-0.83)
CT+TT	46	23			AC+CC	30	9		
					Yes				
Yes CC	9	12	NS		AA	18	13	0.09	0.47(0.17-1.25)
CT+TT	13	23	110^{-6}	6.10(2.43-15.55)	AC+CC	8	16	NS	
Milk consumption <3/week					Milk consumption <3/week				
CC*	52	16	0.002	3.14(1.37-7.26)	AA*	24	35	0.04	0.43(0.17-1.06)
CT+TT	29	28	NS		AC+CC	24	15		
			NS		≥3/week				
≥3/week CC	26	16			AA	16	12	NS	
CT+TT	30	18			AC+CC	14	10	NS	
Cheese<2/week					Cheese<2/week				
CC*	49	27	NS		AA*	20	31	NS	
CT+TT	30	16			AC+CC	23	17		
					≥2/week				
≥2/week CC	29	5	0.02	0.31(0.09-0.98)	AA	20	16	NS	
CT+TT	29	30	NS		AC+CC	15	8	0.03	0.34(0.11-1.07)
Yogurt<2/week					Yogurt<2/week				
CC*	45	22	0.0008	3.94(1.61-9.80)	AA*	23	30	NS	
CT+TT	14	27			AC+CC	18	12		
					≥2/week				
≥2/week CC	33	10	NS		AA	17	17	NS	
CT+TT	45	19	NS		AC+CC	20	13	NS	

P was calculated using CC genotype for MTHFR677 as reference, and AA genotype for MTHFR1298 as reference

Table 8: Interaction between MTHFR polymorphisms with drinking habit and dairy product and colorectal cancer.

MTHFR C677T	Controls(n=137)	Gastric cancer(n=43)	P	OR	MTHFR A1298C	Controls(n=78)	Gastric cancer(n=35)	P	OR
Drinking habit; no					Drinking habit; no				
CC*	69	20	NS		AA*	22	20	0.001	0.18(0.05-0.63)
CT+TT	46	11			AC+CC	30	5		
					yes				
Yes CC	9	4	NS		AA	18	4	0.02	0.24(0.06-0.96)
CT+TT	13	8	NS		AC+CC	8	6	NS	
Milk consumption <3/week					Milk consumption <3/week				
CC*	52	14	NS		AA*	24	12	NS	
CT+TT	29	15			AC+CC	24	8		
					≥3/week				
≥3/week CC	26	10	NS		AA	16	12	NS	
CT+TT	30	4	NS		AC+CC	14	3	NS	
Cheese<2/week					Cheese<2/week				
CC*	49	20	NS		AA*	20	14	NS	
CT+TT	30	12			AC+CC	23	8		
				-	≥2/week				
≥2/week CC	29	4	NS		AA	20	10	NS	
CT+TT	29	7	NS		AC+CC	15	3	NS	
Yogurt<2/week					Yogurt<2/week				
CC*	45	15	NS		AA*	23	10	NS	
CT+TT	14	11			AC+CC	18	6		
					≥2/week				
≥2/week CC	33	9	NS		AA	17	14	NS	
CT+TT	45	8	NS		AC	20	5	NS	

P was calculated using CC genotype for MTHFR677 as reference, and AA genotype for MTHFR1298 as reference

Table 9: Interaction between MTHFR polymorphisms with drinking habit and dairy product and gastric cancer.

Many studies show on the one hand that Calcium in milk is highly bio-available, which may make milk appear to be associated with colorectal cancer risk independent of total calcium intake [43]. Also, other components in milk may contribute to the inverse association. On the other hand calcium in milk may prevent carcinogenesis by reducing colonic cell proliferation.

Clinical trials show that consumption of milk prevents the recurrence of colorectal cancer [48].

Colorectal and gastric cancers are a complex disease due to interaction of genetic and environmental factors; thereby several studies evaluated the gene-environment interaction between MTHFR polymorphisms and gastro-intestinal cancer susceptibility.

According to our result, we found a risk of alcohol intake on GC however frequent intake of milk is protective against CRC furthermore we have undertaken the additive effect of genotype and lifestyle, however there is a significant difference in alcohol intake ($p=110^{-6}$; OR=6.10; 95%CI (2.43-15.55) in CRC group with MTHFR C677T polymorphism if we compare CC genotype of no drinkers versus CT+TT genotype of drinkers, whereas the risk effect of alcohol on GC was eliminated by additive effect, but we found a protective effect of AA genotype in no drinkers compared with drinkers in MTHFR 1298 group. Furthermore we do not observe any association in alcohol intake in GC group as well in both polymorphisms.

The consumption of cheese is also protective in MTHFR677 group and in MTHFR1298 group, however concerning the consumption of yogurt we found that within group who consume less than two times per week the CT+TT genotype presents a risk for CRC this effect was not found in MTHFR1298 group. Furthermore we found that there is no association between several polymorphisms and dairy product in patients with GC. Our finding is in agreement with other studies.

We have grouped clinicopathological characteristics of patients with colorectal and gastric cancer according to their TNM stage, differentiation, lymph nodes and histological type of cancer; furthermore we have analyzed the possible interaction with prognostic factors and genotype distribution. In fact, we have not shown a significant effect of MTHFR polymorphisms on the prognosis of disease. In other studies Differentiation of tumor was found no association with risk of CRC [49], which is in agreement with our study. Moreover all patients were followed up 4 years then we recorded data of surgery and chemotherapy treatment. MTHFR gene has been related to drug metabolism since it is necessary for the synthesis of purines and pyrimidines. When it is deficient it does not only promote cell damage and carcinogenesis, but MTHFR gene also plays a key role in the FU pathway [50]. MTHFR 677 polymorphism results are controversial from the point of view of their role on gastro-intestinal cancer response to 5 FU, Cecchin, et al. found that the T allele was associated with lower response rates in CRC treated by fluoropyrimidine-based chemotherapy [51], however our finding suggested no predictive impact of the MTHFR polymorphisms on tumor response to adjuvant and neo-adjuvant Folfox, Folfiri in combination and monotherapy capecitabine and LV5-FU2. These results are in agreement with other studies [52,53]. Nevertheless MTHFR 1298 was found recently to be a predictive factor for tumor response to folfox (FU-oxaliplatine combination) [54].

In summary, our study shows a risk of MTHFR 677 polymorphism in colorectal cancer, whereas MTHFR 1298 haven't any effect on gastro-intestinal cancers. We also found an association of alcohol consumption with gastro-intestinal cancers and a protective effect of cheese in CRC group.

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