

Journal of Investigative Surgery

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iivs20

Relationship between *MTHFR* Gene Polymorphisms and Gastrointestinal Tumors Development: Perspective from Eastern Part of Turkey

Ersoy Öksüz , Gökhan Görgişen , Gökhan Oto , Hülya Özdemir , Abbas Aras , Murat Öksüz , İsmail Musab Gülaçar & Muhammet Hamdi Demirkol

To cite this article: Ersoy Öksüz , Gökhan Görgişen , Gökhan Oto , Hülya Özdemir , Abbas Aras , Murat Öksüz , İsmail Musab Gülaçar & Muhammet Hamdi Demirkol (2020): Relationship between *MTHFR* Gene Polymorphisms and Gastrointestinal Tumors Development: Perspective from Eastern Part of Turkey, Journal of Investigative Surgery, DOI: 10.1080/08941939.2020.1824249

To link to this article: https://doi.org/10.1080/08941939.2020.1824249

Published online: 15 Dec 2020.

Submit your article to this journal

Article views: 8

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=iivs20

JOURNAL OF INVESTIGATIVE SURGERY
https://doi.org/10.1080/08941939.2020.1824249

Taylor & Francis
Taylor & Francis Group

ORIGINAL RESEARCH

Check for updates

Relationship between *MTHFR* Gene Polymorphisms and Gastrointestinal Tumors Development: Perspective from Eastern Part of Turkey

Ersoy Öksüz[a], Gökhan Görgişen[b], Gökhan Oto[a], Hülya Özdemir[a], Abbas Aras[c], Murat Öksüz[d], İsmail Musab Gülaçar[b], and Muhammet Hamdi Demirkol[e]

[a]Department of Medical Pharmacology, Medical Faculty, Van Yuzuncu Yıl University, Van, Turkey; [b]Department of Medical Biology, Medical Faculty, Van Yuzuncu Yıl University, Van, Turkey; [c]Department of General Surgery, Medical Faculty, Van Yuzuncu Yıl University, Van, Turkey; [d]Department of Gastroenterology, Ortadoğu Hospital, Adana, Turkey; [e]Medical Faculty, Van Yuzuncu Yıl University, Van, Turkey

ABSTRACT

Background: Gastric and esophageal cancers are 2 of the most prevalent cancer types worldwide. Polymorphisms in the genes that code the methylenetetrahydrofolate reductase (MTHFR) enzyme increase the formation of both cancer types. In this study, it was aimed to research the relationship between the existence of *MTHFR C677T* and *A1298C* polymorphisms in patients with gastric and esophageal cancer and the lifespans of patients.

Methods and Materials: This prospective study was performed at Van Yuzuncu Yil University. Included in the study were 30 patients with esophageal tumors, 70 patients with gastric tumors, and 61 healthy volunteers. From each of the patients, 5 mL of blood was drawn. DNA was isolated via kits with spin-column technology.

Results: It was concluded that the risk of developing gastric cancer was 4.13 times higher in individuals who had the *AC* genotype of the *A1298C* polymorphism when compared to those who had the *AA* genotype, while the risk was 2.91 times higher in individuals who had the *CC* genotype when compared to those who had the *AA* genotype ($P = 0.001$, $P = 0.027$). Carriers of the *AC* genotype of the *A1298C* polymorphism had 2.89 times higher risk of developing esophageal cancer when compared to those who had the *AA* genotype ($P = 0.033$). It was determined that individuals who had the 1298 *CC* genotype were not at higher risk of developing esophageal cancer when compared to those with the *AA* genotype ($P = 0.863$). It was concluded that individuals who had the *TT* genotype of the *C677T* polymorphism were not at higher risk of developing gastric and esophageal cancers when compared to those who had the *677CC* genotype ($P > 0.05$). There was no difference in terms of the life spans of the patients with regards to the genotypes ($P > 0.05$).

Conclusion: The results showed that the *A1298C* polymorphism on the MTHFR gene can be a risk factor for gastric and esophageal cancer in eastern Turkey. These polymorphisms may have no effect on the life spans of the patients.

ARTICLE HISTORY
Received 23 May 2020
Revised 20 August 2020
Accepted 12 September 2020

KEYWORDS
Methylenetetrahydrofolate reductase, MTHFR; polymorphism; gastric cancer; esophageal cancer; lifespan

Introduction

Gastric cancer is the fifth most prevalent cancer type in the world. However, it is ranked third for cancer-related deaths [1]. Generally, it is 2 times more prevalent in males than in females and it is more prevalent in the elderly population. Worldwide, it is more common in far eastern countries, such as China and Japan. In Turkey, it is more common in eastern provinces [2,3]. Despite the failure of understanding its complete etiology, it is known that dietary, genetic, and environmental factors are the most important risk factors [4]. Since gastric cancers show their symptoms fairly late, they are in an advanced stage when diagnosed and usually have a bad prognosis. Therefore, realizing early diagnosis methods and new treatment options are quite important.

Esophageal cancer is similar to gastric cancer in many ways. It is more prevalent in males, it shows its symptoms late, and spreads quickly, which leads to a diagnosis at an advanced stage. When all types of cancers are considered, esophageal cancer is the 8th most prevalent. However, it ranks higher in cancer-related deaths due to its high mortality rate. The 5-year survival rate is around 5%–15% [5]. Just like gastric cancer, the prevalence of esophageal cancer shows geographical differences and it is more prevalent in eastern societies [6]. Although diet holds an important position in its etiology, genetic predisposition is also quite important in the formation of esophageal cancer [7].

Methylenetetrahydrofolate reductase (MTHFR), which is the most important enzyme of folate metabolism and consists of 656 amino acids, converts 5,10 methylenetetrahydrofolate (5,10-methylene THF) into 5-methyl tetrahydrofolate (5-methyl THF). 5-methyl THF provides the necessary methyl group for DNA methylation, methionine synthesis,

CONTACT Ersoy Öksüz drugoksuz@hotmail.com Department of Medical Pharmacology, Medical Faculty, Van Yuzuncu Yil University, 65080, Tusba/Van, Turkey.

© 2020 Taylor & Francis Group, LLC

and remethylation of homocysteine. As 5,10-methylene THF converts into 5-methyl THF, it simultaneously oxidizes into 10-formyl THF, which ensures purine synthesis [8]. Any polymorphism or mutation on the gene coding the MTHFR enzyme causes a reduction in the activity of this enzyme. In serious cases of MTHFR enzyme deficiency, substrates in the MTHFR pathways accumulate, nucleic acid synthesis is affected, and hyperhomocysteinemia and homocystinuria occur due to the reduction in the amount of methyl necessary for biochemical events [9]. As a result of that, homeostasis is disrupted, and stroke, thrombosis, peripheral neuropathy, cardiovascular and cerebrovascular diseases are observed [10]. The most prevalent polymorphisms seen on the gene coding the MTHFR enzyme are *C677T* and *A1298C*. The *C677T* polymorphism changes the 677th nucleotide cytosine (C) into→ thymine (T) in the gene coding the MTHFR enzyme. The *A1298C* polymorphism causes the 1298th nucleotide to change from adenine (A) into → cytosine (C) [11]. It has been shown that these polymorphisms cause a predisposition to many types of cancer, including esophageal and gastric cancers, in addition to the diseases mentioned above [12–14].

In addition to the studies showing that polymorphisms in *MTHFR* cause a predisposition to cancer, there have also been studies suggesting that polymorphisms may have a protective effect oncancers, as well as studies suggesting that polymorphisms have no effect. For example, in a meta-analysis evaluating 17,469 cases, it was shown that the risk of acute lymphoblastic leukemia (ALL) formation was lower inindividuals with the *C677T* polymorphism [15]. In a meta-analysis over oral cavity cancer patients, it was determined that the *A1298C* and *C677T* polymorphisms did not form any risk factor [16]. All of these results showed that the effects of MTHFR polymorphisms may show variability depending on the type of cancer and population. Therefore, determining the population-specific effects of MTHFR polymorphisms and discovering their effects on the development of different types of cancers are substantially important.

In this study, it was aimed to determine the existence of *rs1801133/C677T* and *rs1801131/A1298C* polymorphisms on the MTHFR gene of patients with gastric and esophageal tumors in Van Province, Turkey, and investigate the relationship between these polymorphisms and the lifespans of the patients.

Material-method

Ethical approval

Ethical approval for the study was granted by the Van Yuzuncu Yil University Clinical Research Ethics Committee (24.10.2017/decision no.:07)

Study population

Included in this prospective study were 30 patients with esophageal tumors and 70 patients with gastric tumors, who were >18 years of age, resided in Van Province, and were pathologically diagnosed and clinically followed at the Van Yuzuncu Yil University Medical Faculty Hospital General Surgery Clinic, between 2017 and 2019, in addition to 61 healthy volunteers without any family history of gastric or esophageal tumors. Consent of all of the patients and healthy volunteers were taken. Patients with isolated gastric and esophageal tumors were included in the study, while those without consent, with an uncertain diagnosis, and those whose primary tumor was not gastric or esophageal were excluded. Demographic properties, habits, type of malignancy, chemotherapy and radiotherapy history, surgery history, disease start dates, and dates of death of the patients were determined. Dates of death of the patients were taken from the official death notification system used nationwide by scanning with identification number.

DNA isolation and genotyping

In the first phase,5 mL of blood was taken from all of the patients and volunteers. The drawn blood samples were preserved in 4 °C for a short period of time. In the second phase, DNA isolation was achieved with kits that hadspin-column technology. Amount and quality determination of the obtained DNAs was realized using a nanodrop device. DNAs without RNA and protein contamination were preserved at −80 °C until all of the samples were gathered.

Genomic DNA was isolated from peripheral blood mononuclear cells using the GF-1 blood DNA extraction kit (Vivantis Technologies, Malaysia) according to the manufacturer's instructions. Cycle sequencing was used to genotype the *MTHFR* gene polymorphisms. First, DNA fragments that included rs1801131 and rs1801133 regions were amplified by polymerase chain reaction (PCR) reactions using the following primers: exon 4 forward: 5′TGAAGGAGAAGGTGTCTGCGGGA3′, reverse: 5′AGGACGGTGCGGTGAGAGTG3′; exon 7 forward: 5′CTTTGGGGAGCTGAAGG ACTACTAC3′, reverse: 5′-CACTTTGTGACCATTCCGGTTTG-3′. A final reaction volume of 25 µL of PCR contained 100 ng genomic DNA, 10 pmol of each primer, PCR buffer with 1.5 mM of $MgCl_2$, 100 mM of each dNTP, 10% dimethylsulfoxide and 0.5 U of Taq polymerase (Thermo Scientific, MA, USA). PCR was performed on a ABI Veritithermal cycler (Thermo Scientific) using the following conditions: 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, 59°C for 1 min, and 72°C for 45 s, and a final extension at 72°C for 5 min.

PCR products were purified by the ExoSap reaction and the Big dye-terminator sequencing kit (Applied Biosystems, CA, USA) was used for the primers during amplification. Sequencing fragments were detected by capillary electrophoresis using the ABI Prism 3130 DNA analyzer (Applied Biosystems). Sequence chromatograms were analyzed by Finch TV (Figures 1,2).

Statistical data analysis

Data were summarized as the mean ± standard deviation for continuous variables, and frequencies (percentiles) for categorical variables. Student t test was used for independent

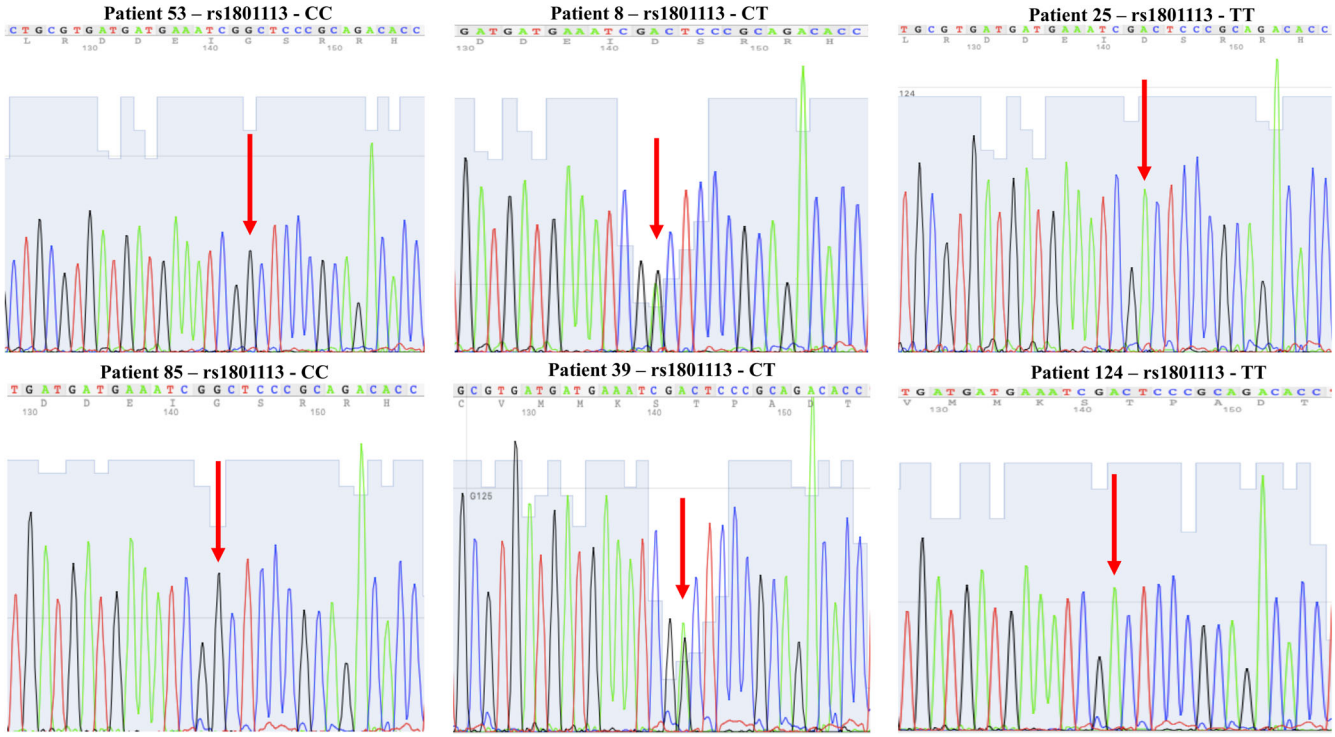


Figure 1. Representative results for the sequencing analysis of the MTHFR gene rs1801113 polymorphism.

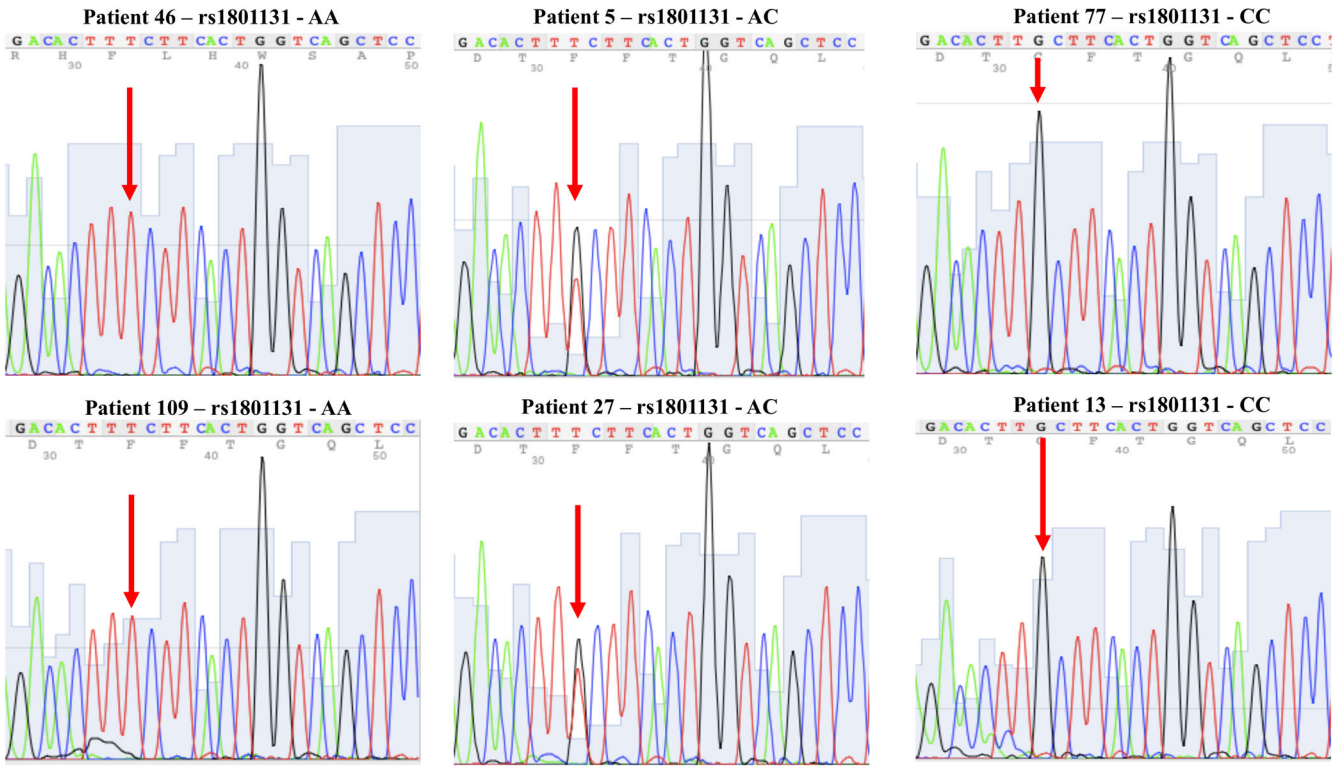


Figure 2. Representative results for the sequencing analysis of the MTHFR gene rs1801131 polymorphism.

patient and control group comparisons. Chi square test was used for proportions and its counterpart, the Fisher exact test, was used when the data were sparse. The survival estimations were performed using the method of the Kaplan-Meier algorithm and the mean survival time (lifespan) was calculated with the related standard error. The Tarone-Ware test was used to compare the overall survival times between the groups depending on the pattern of censoring. The association between genes focused on being gastric or esophageal cancer and was evaluated by multiple logistic regression analysis. Odds ratios and their confidence intervals were calculated. Cox proportional hazard model analyses were used to

Table 1. Demographic properties of the patients and healthy volunteers.

Patients	Female (n:50/50) n/%	Male (n:50/50) n/%	Total (n:100) n/%
Age ≤60	19/38	23/46	42/42
Age >60	31/62	27/54	58/58
Mean Age	63.04	63.15	63
Gastric cancer	32/46	38/54	70/70
Esophageal cancer	18/60	12/40	30/30
Surgery	28/56	22/44	50/50
Chemotherapy	15/30	13/26	28/28
Radiotherapy	6/12	7/14	13/13
Family history	17/34	12/24	29/29
Smoking	13/26	21/42	34/34
Alcohol	0/0	5/10	5/5
Healthy volunteers	Female (n:31/51) n/%	Male (n:30/49) n/%	Total (n:61) n/%
Age ≤ 60	12/39	12/40	24/39
Age > 60	19/61	18/60	37/61
Mean Age	64.2	62	63.78

Table 2. Gender/age evaluation of the patients and healthy volunteers, and evaluation of the mean age of the patients.

Variables		Patient (n,%)	Control(n,%)	P-value
Gender	Male	50 (%63.3)	29 (%36.7)	0.871
	Female	50 (%61.0)	32 (%39.0)	
Age	≤60	42 (%63.6)	24 (%36.4)	0.745
	>60	58 (%61.1)	37 (%38.9)	
Patients	Mean age/ mean ± SD	n		P-value
Esophageal ca	61.63 ± 11.90	30		
Gastric ca	63.64 ± 13.60	70		0.733
Control	63.78 ± 12.87	61		

Table 3. Rs1801133/C677T polymorphism in the MTHFR gene for the gastric cancer patients and healthy volunteers.

	B	Standard error	Odds ratio	95% CI Low	95% CI High	P-value
CC						
CT	0.19	0.40	1.21	0.54	2.67	0.635
TT	0.02	0.51	1.02	0.37	2.80	0.961

determine the effects of the genotypes on mortality. $P < 0.05$ was considered statistically significant and SPSS 15.0 for Windows (IBM Corp., Armonk, NY, USA) was used for all of the statistical analyses.

Results

Demographic properties of the patients in the study are given in Table 1. Of the patients, 50 were female (50%), 50 were male (50%), and the mean age was 63 years. Of the healthy volunteers, 31 were female (51%), 30 were male (49%), and mean age was 63.78 years. All of the gastric cancers were adenocarcinoma and nearly all of the esophageal tumor patients had squamous cell carcinomas (SCCs), which were pathologically classified.

There was no statistically significant difference between the patients and healthy volunteers in terms of gender, age, and mean age (Table 2).

When the patients with gastric cancer and healthy volunteers were evaluated, it was determined that individuals with the *CT* or *TT* genotype of *C677T* had no increased risk of developing gastric cancer when compared to individuals with the *CC* genotype ($P = 0.633$, $P = 0.961$, respectively) (Table 3). The study revealed that the risk of developing gastric cancer was 4.13 times higher in individuals who had the *AC* genotype of *C1298A* when compared to those who had the *AA* genotype, and this risk was found to be statistically significant ($P = 0.001$). Additionally, it was determined that the risk of developing gastric cancer was 2.91 times higher in individuals who had the *CC* genotype when compared to those who had the *AA* genotype ($P = 0.027$) (Table 4, Figure 1).

Table 4. Rs1801131/A1298C polymorphism in the MTHFR gene for the gastric cancer patients and healthy volunteers.

	B	Standard error	Odds ratio	95% CI Low	95% CI High	P-value
AA						
AC	1.41	0.42	4.13	1.81	9.43	**0.001**
CC	1.07	0.48	2.91	1.12	7.51	**0.027**

Table 5. Rs1801133/C677T polymorphism in the MTHFR gene for the esophageal cancer patients and healthy volunteers.

	B	Standard error	Odds ratio	95% CI Low	95% CI High	P-value
CC						
CT	0.57	0.58	0.56	0.17	1.76	0.324
TT	0.00	0.62	1.00	0.29	3.39	1.000

Table 6. Rs1801131/A1298C polymorphism in the MTHFR gene for the esophageal cancer patients and healthy volunteers.

	B	Standard error	Odds ratio	95% CI Low	95% CI High	P-value
AA						
AC	1.06	0.49	2.89	1.08	7.69	**0.033**
CC	0.11	0.67	1.12	0.29	4.25	0.863

Similar to gastric cancer, it was determined that having the *CT* or *TT* genotype of *C677T* did not increase the risk of developing esophageal cancer when compared to the *CC* genotype ($P = 0.324$, $P = 1.000$, respectively) (Table 5). Again, similar to gastric cancer, the risk of developingesophageal cancer was 2.89 times higher in individuals who had the *AC* of *C1298A* genotype when comparedto those who had the *AA* genotype, and this risk was statistically significant ($P = 0.033$). Differing from gastric cancer, it was determined that having the *CC* genotype did not increase the risk of developing esophageal cancer when compared to the *AA* genotype ($P = 0.863$) (Table 6, Figure 2).

Considering the controls separately from the patients with gastric cancer and esophageal cancer, the genotype frequency of *C677T* was 59% for *CC*, 26.2% for *CT* and 14.8% for *TT* and the genotype frequency of *A1298C* was 55.7% for *AA*, 26.2% for *AC* and 18% for *CC*.

According to the result of gastric cancer patients, the frequencies of *CC*, *CT* and *TT* genotypes of *C677T* were 55.7%, 30% and 14.3% respectively. In the same patient group, we detected the genotype frequencies of *A1298C* as 36.7%, 50% and 13.3% for *AA*, *AC* and *CC* genotypes, respectively. Our results showed that *AC* genotype frequency of *A1298C*

Table 7. Genotype distributions of MTHFR gene polymorphisms in control, gastric cancer and esophageal cancer patients.

	n, (%)	Control	Gastric cancer	p*
	CC	36 (59.0)	39 (55.7)	
C6777T	CT	16 (26.2)	21 (30.0)	0.883
	TT	9 (14.8)	10 (14.3)	
	AA	34 (55.7)	18 (25.7)	
A1298C	AC	16 (26.2)	35 (50.0)	0.002
	CC	11 (18.0)	17 (24.3)	
	n, (%)	Control	Esophageal cancer	p*
	CC	36 (59.0)	20 (66.7)	
C6777T	CT	16 (26.2)	5 (16.7)	0.629
	TT	9 (14.8)	5 (16.7)	
	AA	34 (55.7)	11 (36.7)	
A1298C	AC	16 (26.2)	15 (50.0)	0.085
	CC	11 (18.0)	4 (13.3)	

*Fisher's Exact p value.

Table 8. Lifespans of the deceased patients according to their rs1801133/C677T genotypes.

		Mean lifespan/ month	Standard error	95% CI		
Diagnosis	Rs1801133			Low	High	P-value
Gastric ca	CC	27.11	2.74	21.73	32.48	0.111
	CT	29.68	3.66	22.50	36.87	
	TT	16.62	5.46	5.90	27.34	
	Total	26.40	2.11	22.26	30.54	
Esophageal ca	CC	11.40	2.18	7.12	15.67	0.587
	CT	21.33	7.07	7.46	35.20	
	TT	12.80	3.98	4.99	20.60	
	Total	13.44	2.19	9.15	17.73	

polymorphism was significantly higher in gastric cancer patients compared to the control group (P = 0.002) (Table 7).

In esophageal cancer patients, the frequencies of *CC, CT* and *TT* genotypes of *C677T* were 66.7%, 16.7% and 16.7%, respectively. The genotype frequencies of *A1298C* polymorphism in the same patient group were detected as 36.7% for *AA*, 50% for *AC* and 13.3% for *CC*. We did not observe any significant difference between esophageal cancer and control group in genotype distributions of both polymorphisms (Table 7).

For a multivariate logistic regression model with both genotypes included, it was observed that the *C6777T* polymorphism was ineffective when the *A1298C* polymorphism is present. The same situation was also observed for gastric cancer. In conclusion, the *C6777T* polymorphism had no statistical significance for the risk of developing gastric and esophageal cancer.

There was no statistically significant difference between the *CT* and *TT* genotypes, and *CC* genotype, and between the *AC* and *CC*, genotypes, andthe *AA* genotype in terms of the mean lifespan of the patients with gastric and esophageal cancer (P = 0.111, P = 0.587, P = 0.969, P = 0.689, respectively) (Tables 8 and 9; Figures 3a–b, 4a–b).

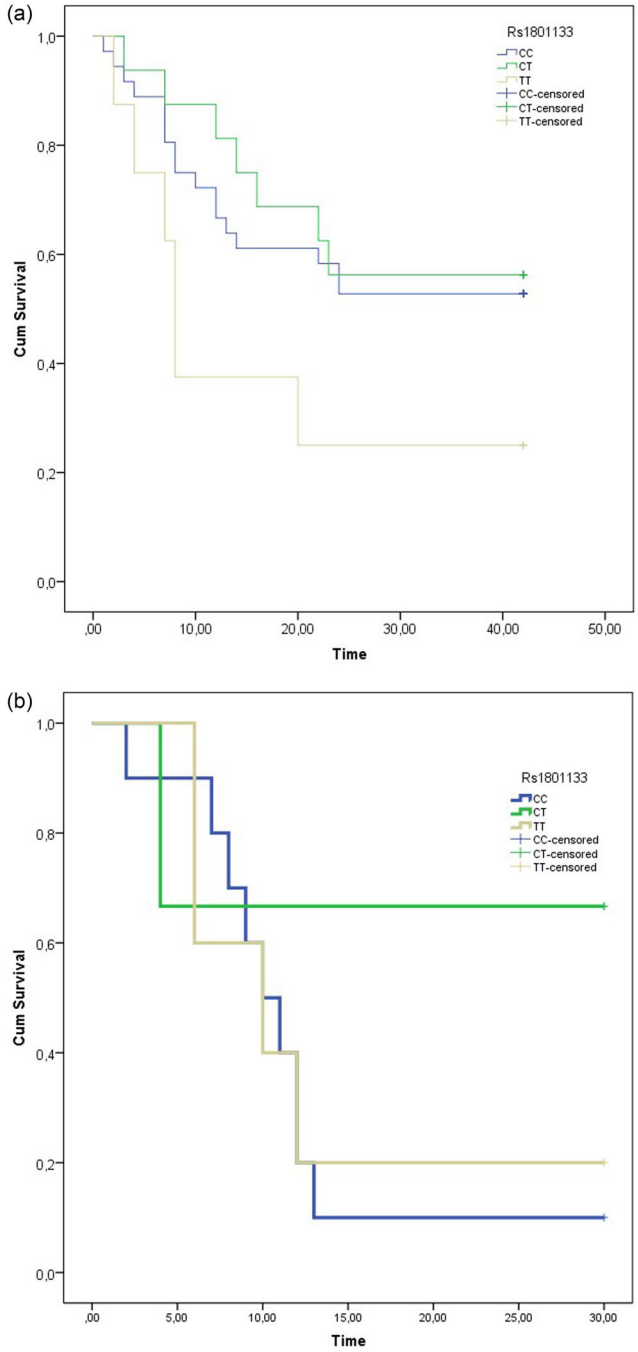


Figure 3. Lifespans of the deceased patients according to their rs1801133/C677T genotype. **(a).** Gastric ca rs1801133/C677T, **(b)**. Esophageal ca; Rs1801133/C677T (time: month).

Discussion

Gastric cancer holds an important position among all types of cancer due to its high prevalence, as well as the fact that it is responsible for approximately 10% of cancer-related deaths. However, in recent years, its prevalence has decreased notably, especially in regions where it is seen commonly, such as Japan, due to the implementation of early diagnosis methods. Smoking, alcohol, diets that comprise foods that are too salty and fatty with low amounts of vegetables and fruits, lower socioeconomic status, obesity, pernicious anemia, and Helicobacter pylori (HP) infection are the most important risk factors [17]. HP infection is common worldwide and especially causes distal-type gastric cancer [18]. In addition to all of these well-known risk factors, genetic predisposition, such as an increase in the activation of miRNAs, which is responsible for tumor formation, prognosis, and metastasis, and intracellular

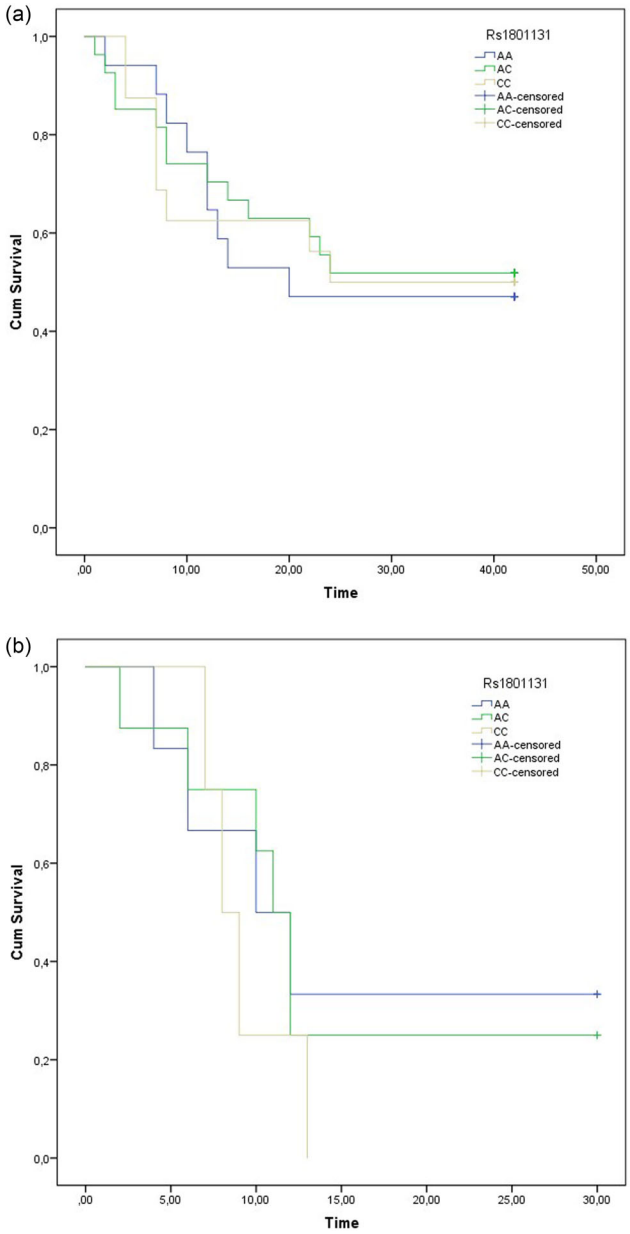


Figure 4. Lifespans of the deceased patients according to their rs1801133/A677C genotypes. **(a).** Gastric ca rs1801131/A1298C, **(b).** Esophageal ca rs1801131/A1298C.

signaling pathways are other risk factors that have been particularly drawing attention. The reason genetic predisposition has been drawing attention is the fact that the prevalence of gastric cancer is particularly low in regions where HP infections are prevalent [19]. It has been determined in various genetic studies that single nucleotide polymorphisms (SNPs) and mutations in several genes responsible for HP, carcinogen metabolism, tumor suppression, deoxynucleotide synthesis, and DNA repair are risk factors for gastric cancer [17]. One the most important of these is SNPs on the gene coding the MTHFR enzyme responsible for deoxynucleotide synthesis. Although there have been many studies conducted indifferent parts of the world showing that SNPs on the gene coding the MTHFR enzyme are a risk factor for the formation of gastric cancer,

Table 9. Lifespans of the deceased patients according to their irs1801131/A1298C genotypes.

Diagnosis	Rs1801131	Mean lifespan/ months	Standard error	95% CI		P-value
				Low	High	
Gastric ca	AA	25.52	3.85	17.96	33.09	0.969
	AC	27.07	3.16	20.87	33.27	
	CC	26.18	4.16	18.02	34.35	
	Total	26.40	2.11	22.26	30.54	
Esophageal ca	AA	15.33	4.36	6.78	23.88	0.689
	AC	14.12	3.43	7.40	20.84	
	CC	9.25	1.31	6.67	11.82	
	Total	13.44	2.19	9.15	17.73	

there have also been studies with different results. For example, in a study conducted in Italy, it was determined that the *TT* genotype of the MTHFR *C677T* polymorphism caused a predisposition to gastric cancer and the *A1298C* polymorphism did not increase the risk factor [20]. In a study conducted in northwestern China on 681 patients with gastric cancer, it was determined that the *C677T* polymorphism is a risk factor for gastric cancer [21]. In the meta-analysis on gastric cancers in western and eastern societies, it was determined that the MTHFR *C677T* polymorphism is a worldwide risk factor for gastric cancer and contrarily, it was determined that the *A1298C* polymorphism is a protective factor in eastern societies [22]. In a meta-analysis, it was determined that the *C677T* polymorphism did not increase the risk for gastric cancer in Caucasian populations; however, it did weakly increase the risk factor for Asian populations, contrary to the studies mentioned above [12]. In a study conducted in Korea, it was determined that the *C677T* and *A1298C* polymorphisms did not increase the risk factor for gastric cancer, contrary to the studies showing that SNPs on the MTHFR gene cause cancer [23]. In another study conducted in Korea, it was determined that the *677CT* polymorphism was a protective factor against gastric cancer [24]. Hence, the current study is quite important due to the fact that it is the first study, as far as is known, to investigate the effects of the *MTHFR* polymorphisms on gastric cancer, which is seen more commonly in the eastern region of Turkey. The findings of thisstudy showed that the risk of developing gastric cancer was 4.1 times higher when the *rs1801131/A1298C* genotype was present when compared to the *AA* genotype ($P = 0.001$). The risk was 2.9 times higher with the *rs1801131/C1298C* genotype ($P = 0.027$). Contrary to other studies, the *C677T* polymorphism did not increase the risk of developing gastric cancer. Again, another important aspect of this study was suggesting, for the first time, that the *A1298C* polymorphism may be a risk factor in the formation of gastric cancer, contrary to the studies mentioned above. As it can be inferred from the studies mentioned above, the effects of the MTHFR gene polymorphisms on cancer can be fairly varied between different populations. Even so, as that was the case in the current study, the existence of the polymorphism may be a risk factor for one population while protective for another. This difference can be caused by racial differences, as well as the existence of HP infection, dietary habits, alcohol, smoking, environmental differences, or different SNPs on the MTHFR and other genes. However, the results of the

current study showed that the reason that *A1298C* is a risk factor was more likely to be racial differences, rather than dietary or other habits. For example, family history was too common to be ignored (34% female, 24% male), and alcohol usage, which is an important risk factor for gastric cancer, was quite low (0% female, 24% male). Similarly, the smoking rate was not particularly high (26% female, 24% male). Another different finding of this study was the fact that the difference between the prevalence of gastric cancer in males and females was quitelow, contrary to the worldwide statistic showing that males are twice as likely to have gastric cancer when compared to females (n: 38, n:32, respectively). Different studies conducted country-wide that analyze all of these data, are necessary.

The prevalence of esophageal cancer in southern and eastern Africa and eastern Asia is nearly 16 times higher when compared to western societies, central Africa, and central America. In Turkey, it is more common in the eastern region which includes Van Province, and the same is true for gastric cancers. It is 3–4 times more common in males when compared to females worldwide [25]. The most common type of esophageal cancer is SCCs, which often forms in the middle-upper esophagus and is generally common in eastern regions [26]. Although its etiology is not exactly known, diets with a low amount of fresh vegetables and fruits, and hot liquid intake (tea/coffee) are the most important risk factors for eastern populations [27]. The most important risk factors for western populations, which have low prevalence for esophageal cancers, are smoking and alcohol consumption. Obesity, gastroesophageal reflux, smoking, and Barrett esophagus are other risk factors [28]. In addition to all of these risk factors, genetic disposition has an important role in esophageal cancer as it also does ingastric cancer. In a study conducted in China, it was determined that variations on DNA repair pathways are a risk factor for esophageal cancer [29]. In another study, it was determined that SPNs on many different genes are an important risk factor [30]. In addition to other genes, there are many studies that have researched the SPNs on the MTHFR coding gene and its effects on esophageal cancer. In the aforementioned study conducted in Italy, it was determined that the *TT* genotype of the *C677T* polymorphism caused a predisposition to esophageal cancer, similar to gastric cancer, and the *A1298C* polymorphism did not increase the risk factor [20]. In another study conducted in China, it was determined that the *C677T* polymorphism is a risk factor for SCCs [31]. In a study conducted in northern China, it was shown that the *TT* genotype of the *C677T* polymorphism was a risk factor for SCCs [32]. As far as is known, the current study is the first that researched the relationship between esophageal cancer and gastric cancer, and the MTHFR polymorphisms in the eastern region of Turkey. This is especially important due to the fact that not only is this the first study, but it also provides different results than the other studies in the literature. In the current study, it was determined that the *C677T* genotype did not increase the risk of developing esophageal cancer like it does for gastric cancer, contrary to the studies conducted in other parts of the world. The risk of developing esophageal cancer was 2.9 times higher in individuals who had the *A1298C* genotypewhen compared to the *AA* genotype, similar to that for gastric cancer ($P = 0.033$). However, it was determined that having the 1298 *CC* genotype didnot increase the risk of developing esophageal cancer, which differedfrom gastric cancer ($P = 0.863$). Although the statistical significance seen in heterozygote cases like *AC* was expected to be seen in homozygote cases like *CC*, it was not observed in the current study. This situation may have been caused by the low number of esophageal cancer patients. This result must be reinforced with studies done on a large number of patients with esophageal tumors. The fact that the situation observed on esophageal cancer patients was more or less similar to that of the gastric cancer patients supported the idea that racial differences unique to the region may have had a significant contribution on cancer formation. Compatible with the literature, almost all of the esophageal cancer patients had SCCs. The diet style, which is a risk factor for SCCs, is a common diet style on this region, such as vegetable and fruit consumption is low in theregion. Again, the habit of drinking hot tea, which is another risk factor, is very common. And again compatible with the literature, the amount of patients with adenocarcinoma related to alcohol and smoking, which is very prevalent in western regions, was nearly non-existent (n:1). In addition to that, in thisstudy, the number of females was higher than males, despite the fact that esophageal cancer is more common in males when compared to females worldwide (n:18, n:12, respectively). This situation may have been caused from the fact that dietary habits, like drinking hot tea, was more common in females. In the meantime, the tradition of cooking bread over awood fire was significantly common for the females on theregion and this situation may have been a risk factor for esophageal cancer. Again, unique to the gender effect on esophageal cancer, the activity of SNPs may have been increased or the females may have had an additional, different SNP. More studies are necessary to reveal all of these effects.

There have also been publications researching the effects of SNPs on the MTHFR gene on the lifespan of cancer patients. In a study conducted on patients with glioblastoma, it is determined that the lifespan for the *C677T* polymorphism *TT* genotype was shorter when compared to the other genotypes and the *A1298C* genotype hadno effect on lifespan [33]. In a study conducted in China on patients with gastric cancer, it was determined that patients with the *A1298C* polymorphism hada longer lifespan [34]. The findings herein were different from these 2 studies. Neither of the polymorphisms had any effect on the lifespan of the patients with gastric and esophageal cancer ($P = 0.111$, $P = 0.587$, $P = 0.969$, and $P = 0.689$, respectively). However, only the disease start dates and death dates of the patients were recorded in the current study. Therefore, it should not be forgotten that the ages of the patients, stage of the tumor when diagnosed, existence of metastasis, or existence of additional diseases might affect the lifespan.

Conclusion

The findings of the current study showed that the *A1298C* polymorphism on the MTHFR gene may be a risk factor for gastric and esophageal cancer in Van Province in Turkey, and the *C677T* polymorphism hadno effect on either of the cancers. The findings of the study asserted that SNPs may have no effect on the lifespan of patients suffering from either types of cancer. However, in our study, the number of patients is low and comprehensive studies involving larger patients are needed.

Acknowledgments

We would like to thank to Zihni Yigit Oksuz from University British Columbia for his contribution to the experimental procedure.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Authors' contributions

E.Ö. designed the study, collected and analyzed the data, wrote the manuscript. G.G. and I.M.G. performed the experimental procedure, G.O., H.Ö, A.A., M.Ö. and M.H.D reviewed the manuscript and contributed to data collection. All authors critically revised the manuscript, approved the final version to be published and agree to be accountable for all aspects of the work.

Funding

This work was supported by Van Yuzuncu Yil University Scientific research projects (TSA-2018-6664). We declared that there is no conflict of interest with funding situations.

ORCID

Ersoy Öksüz http://orcid.org/0000-0002-8088-1009
Gökhan Görgişen http://orcid.org/0000-0001-6040-7863
Gökhan Oto http://orcid.org/0000-0001-7310-7800

References

1. Ren W, Li W, Wang D, Hu S, Suo J, Ying X. Combining multi-dimensional data to identify key genes and pathways in gastric cancer. *Peer J*. 2017;5:3385–3302. doi:10.7717/peerj.3385.
2. Wayman J, Forman D, Griffin SM. Monitoring the changing pattern of esophago gastric cancer: data from a UK regional cancer registry. *Cancer Causes Control*. 2001;12(10):943–949. doi:10.1023/A:1013756531219.
3. Yalçın B, Zengin N, Aydın F, et al. The clinical and pathological features of patients with gastric cancer in Turkey: a Turkish oncology group study. *Turkish J Cancer*. 2006;36:108–111.
4. Alacalı M. Gastric cancer, screening and prevention methods. *Ankara Med J*. 2012;12:195–198.
5. Arnal MJD, Arenas ÁF, Arbeloa ÁL. Esophageal cancer: risk factors, screening and endoscopictreatment in western and eastern countries. *WJG*. 2015;21(26):7933. doi:10.3748/wjg.v21.i26.7933.
6. Altorki N, Kent M, Ferrara C, Port J. Results of nationwide study on the three field lymph node dissection of esophageal cancer. *Oncology*. 1991;48:411–420. doi:10.1097/01.SLA.0000021583.51164.F4.
7. Shen GR, Li WZ, Liu YC, Li XP, Yuan HY. Association between a microRNA-214 binding site polymorphism in the methylenetetrahydrofolate reductase gene and esophageal squamous cell carcinoma. *Genetic Mol Res*. 2016;15:1–6. doi:10.4238/gmr.15028158.
8. Bagley PJ, Selhub J. A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. *Proc Natl Acad Sci USA*. 1998;95(22):13217–13220. doi:10.1073/pnas.95.22.13217.
9. Dikmen M. Molecular biology of methylenetetrahydrofolate reductase (MTHFR) enzyme and its association with diseases. *Med J Kocatepe*. 2004;5:9–16.
10. Rezende LM, Augusto F, Marson L, Sílvia C, Lima P, Bertuzzo CS. Can MTHFR C677T and A1298C polymorphisms alter the risk and severity of sporadic breast cancer in Brazilian women? *Clin Breast Cancer*. 2017;1:1–8. doi:10.1016/j.clbc.2017.02.004.
11. Schneider JA, Rees DC, Liu YT, Clegg JB. Worldwide distribution of a common methylenetetrahydrofolate reductase mutation. *Am J Hum Genet*. 1998;62(5):1258–1260. doi:10.1086/301836.
12. Chen L, Lu N, Zhang BH, Weng L, Lu J. Association between the MTHFR C677T polymorphism and gastric cancer susceptibility: A meta-analysis of 5,757 cases and 8,501 controls. *Oncol Lett*. 2015;10(2):1159–1165. doi:10.3892/ol.2015.3356.
13. Arslan S, Karadayi S, Yildirim ME, Özdemir Ö, Akkurt İ. The association between methylene-tetrahydrofolate reductase gene polymorphism and lung cancer risk. *Mol Biol Rep*. 2011;38(2):991–996. doi:10.1007/s11033-010-0194-z.
14. Piyathilake CJ, Macaluso M, Johanning GL, Whiteside M, Heimburger DC, Giuliano A. Methylenetetrahydrofolate reductase (MTHFR) polymorphisim increases the risk of cervical intraepithelial neoplasia. *Anticancer Res*. 2000;20:1751–1757.
15. Zhang B, Zhang W, Yan L, Wang D. The association between MTHFR gene C677T polymorphismand ALL risk based on a meta-analysis involving 17,469 subjects. *ClinicaChimicaActa*. 2017;466:85–82. doi:10.1016/j.cca.2017.01.001.
16. Jiang S, Xu JD, Zhuo ZJ, Hua ZM. Association of MTHFR c677T and a1298c polymorphisms with oral cancer susceptibility: evidence from a meta-analysis. *Onco Targets Ther*. 2017;10:303–310. doi: 10.2147/OTT.S124690
17. Wadhwa R, Song S, Lee JS, Yao Y, Wei Q, Ajani JA. Gastric cancer-molecular and clinical dimensions. *Nat Rev Clin Oncol*. 2013;10(11):643–655. doi: 10.1038/nrclinonc.2013.170.
18. Conteduca V, Sansonno D, Lauletta G, Russi S, Ingravallo G, Dammacco FH. Pylori infection and gastric cancer: state of the art (review). *Int J Oncol*. 2013;42(1):5–18. doi:10.3892/ijo.2012.1701.
19. Holcombe C. Helicobacter pylori: The African enigma. *Gut*. 1992;33(4):429–431. doi:10.1136/gut.33.4.429.
20. Mazzuca F, Borro M, Botticelli A, et al. Effect of MTHFR Polymorphisms on gastrointestinal cancer risk in Italy. *World J Oncol*. 2015;6(4):394–397. doi:10.14740/wjon930w.
21. Wei L, Niu F, Wu J, et al. Association study between genetic polymorphisms in folate metabolism and gastric cancer susceptibility in Chinese Han population: a case control study. *Mol Genet Genomic Med*. 2019;7(5):e633. doi:10.1002/mgg3.633.
22. Xia LZ, Liu Y, Xu XZ, et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancer susceptibility. *World J Gastroenterol*. 2014;20(32):11429–11438. doi:10.3748/wjg.v20.i32.11429.
23. Kim JK, Kim S, Han JH, et al. Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of stomach cancer in a Korean population. *Anticancer Res*. 2005;25:2249–2252.
24. Cui LH, Shin MH, Kweon SS, et al. Methylenetetrahydrofolate reductase C677T polymorphism in patients with gastric and colorectal cancer in a Korean population. *BMC Cancer*. 2010;10:236–234. doi:10.1186/1471-2407-10-236.
25. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global Cancer statistics. *CA Cancer J Clin*. 2011;61(2):69–79. doi:10.3322/caac
26. Jemal A, Center MM, Desantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. *Cancer*

Epidemiol Biomarkers Prev. 2010;19(8):1893–1907. doi:10.1158/1055-9965.EPI-10-0437.

27. Islami F, Boffetta P, Ren JS, Pedoeim L, Khatib D, Kamangar F. High-temperature beverages and foods and esophageal cancer risk-a systematic review. *Int J Cancer*. 2009;125(3):491–524. doi:10.1002/ijc.24445.
28. Engel LS, Chow WH, Vaughan TL, et al. Population attributable risks of esophageal and gastric cancers. *J Natl Cancer Inst*. 2003;95(18):1404–1413. doi:10.1093/jnci/djg047.
29. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. *J Nutr*. 2000;130(2):129–132. doi:10.1093/jn/130.2.129.
30. Ye B, Feng J, Pan X, et al. Genetic variant of single-nucleotide polymorphism is associated with risk of esophageal squamous cell carcinoma. *Genet Test Mol Biomarkers*. 2014;18(1):45–49. doi:10.1089/gtmb.2013.0336.
31. Rachael Z, Solomon S, Qiao YL, et al. Esophageal and gastric cardia cancer risk and Folate- and Vitamin B12-related polymorphisms in Linxian, China. *Cancer Epidemiol Biomark Prevent*. 2003;12:1222–1226.
32. Pan D, Su M, Huang G, et al. MTHFR C677T genetic polymorphism in combination with serum vitamin B2, B12 and aberrant DNA methylation of P16 and P53 genes in esophageal squamous cell carcinoma and esophageal precancerous lesions: a case-control study. *Cancer Cell Int*. 2019;19:288–280. doi:10.1186/s12935-019-1012-x.
33. Linnebank M, Semmler A, Moskau S, Smulders Y, Blom H, Simon M. The methylenetetrahydrofolate reductase (MTHFR) variant c.677C > T (A222V) influences overall survival of patients with glioblastoma multiforme. *Neuro-oncology*. 2008;10(4):548–552. doi:10.1215/15228517-2008-020.
34. Zhao T, Gu D, Xu Z, et al. Polymorphism in one-carbon metabolism pathway affects survival of gastric cancer patients: large and comprehensive study. *Oncotarget*. 2015;6(11):9564–9576. doi:10.18632/oncotarget.3259.