

RESEARCH ARTICLE

Lack of Association between the MTHFR C677T Polymorphism and Lung Cancer in a Turkish Population

Meral Yilmaz^{1*}, Turgut Kacan², Ismail Sari³, Saadettin Kilickap⁴

Abstract

Background: In this case-control study, we aimed to investigate the relationship between the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and lung cancer. **Materials and Methods:** Total 200 individuals including 100 patients with lung cancer and 100 controls were analyzed. Genotyping of MTHFR C677T was performed using PCR and RFLP methods. **Results:** The majority of the patients were men and 90% were smokers. We found that the risk ratio for development of LC was 13-times higher in smokers compared with non-smokers between patient and control groups in our study (OR:13.5, 95% CI:6.27-29.04, p:0.0001). Besides, the risk ratio for development of LC was nine times higher in individuals with cancer history in their family than those without cancer history (OR:9.65, 95% CI: 2.79-33.36; p:0.0001). When genotype distributions and allele frequencies were analyzed in the study groups, no significant difference was apparent (χ^2 :0.53, p=0.76). In addition, no correlation between genotypes of MTHFR C677T polymorphism and histological type of LC was found (χ^2 :0.99, p=0.60). **Conclusions:** These results suggest that there was no association between the MTHFR C677T polymorphism and lung cancer in the Turkish population.

Keywords: Lung cancer - risk factor - methylenetetrahydrofolate reductase gene - polymorphism.

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Introduction

Lung cancer (LC) is the most common cancer in the world and is the leading cause of cancer-related death in both men and women (Jemal et al., 2011; Siegel et al., 2013). Non-small cell lung cancer (NSCLC) accounts for about 80% of all lung cancers and 15% are small cell lung cancer (SCLC) (Barry et al., 2007). Patients with LC are diagnosed in advanced stage and the prognosis is still poor (Jemal et al., 2011). Smoking is the most important risk factor for LC, but it induces only 10-20% of lifetime smokers to develop LC (Shields, 2002; Hou et al., 2012). Genetic susceptibility and dietary habits may be the main reason for this variation when exposed to same risk factors (Hou et al., 2012). Numerous epidemiological studies have showed that consumptions of vegetables and fruits are associated with the reduced risk for LC (Voorrips et al., 2000; Shen et al., 2003). Folate is one of the constituents found in vegetables and has a protective effect on development of cancer (Choi and Mason, 2000; Voorrips et al., 2000). It plays a critical role on syntheses of nucleotides and DNA repair. Low folate intake levels was shown to lead chromosomal DNA damage, DNA strand breaks, impaired DNA repair and DNA hypo-methylation (Duthie, 1999).

Methylenetetrahydrofolate reductase (MTHFR) is a fundamental enzyme, which catalyzes the 5,10- MTHF to 5-MTHF. Reduced activity of MTHFR may lead to DNA hypo-methylation and also a reduced level of MTHFR substrate may lead to diminished DNA repair and increased frequency of chromosomal breaks and damage (Krajinovic et al., 2004). Therefore, MTHFR may play a critical role in aetiology of LC in combination with other risk factors. The most common mutation of MTHFR is a point mutation (C→T substitution at nucleotide 677) which decreases the activity of MTHFR enzyme (Mtiraoui et al., 2006). The C677T polymorphism occurs in exon 4 and at codon 222 (Robien and Ulrich, 2003).

In this study, we aimed to investigate the relationship between MTHFR C677T polymorphism and LC in a Turkish population.

Materials and Methods

Study group

The research is a case-control study. The one hundred patients with LC who were diagnosed and followed-up at the Department of Medical Oncology at Cumhuriyet University Hospital in Turkey in the year of 2012 and 2013. As a control group, total 100 individuals were

¹Department of Research Centre, ²Department of Medical Oncology, ³Department of Medical Biochemistry, Faculty of Medicine, Cumhuriyet University, Sivas, ⁴Department of Medical Oncology, Faculty of Medicine, Hacettepe University, Ankara, Turkey *For correspondence: meralylmz@gmail.com

selected from among healthy voluntary individuals whose age and gender matched with case group. Both cases and controls accepted to participate to this research and signed informed consent form. The study was approved by The Local Ethical Committee of The University (The Decision Number; 2011/024).

DNA extraction

Whole peripheral blood samples were collected into EDTA-containing tubes from the study population. The salting out method was used for DNA extraction. DNA samples were stored at -20°C until analyzed.

Genotyping

Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods used for genotyping of MTHFR polymorphism. The specific MTHFR gene region concerning C677T single nucleotide polymorphism (SNP) was amplified by using a primer set (Forward primer: 5'-CAA AGG CCA CCC CGA AGC -3'; reverse primer: 5'-ACG GTG CGG TGA GAG TG -3'). PCR was performed on an thermal cycler (Applied Biosystems Gene AmpR PCR system 9700, USA) in a reaction volume of 25 µL containing 10mmol/L Tris-HCl (pH 8.3 at 25°C), 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 2.5 U of Taq DNA polymerase (Fermentas), 5 nmol each of four deoxynucleotide triphosphates (dNTPs-Fermentas), 10 pmol each of the appropriate primers and 100 ng of genomic DNA. PCR conditions were one cycle of 94°C for 5 minute (min); 35 cycles of 94°C for 30 second (s), 58°C for 45 s, 72°C for 40 s, and a final cycle of 72°C for 10 min.

A fragment with 246 base pair (bp) was obtained after PCR amplification. The PCR product was digested with HinfI restriction endonuclease (RE) enzyme (5U/µL-Fermentas) in a total reaction volume of 20 µL including 1X FB buffer for overnight at 37°C. PCR and RFLP products were separated on 2.5% agarose gels. Agarose gels were imaged on UV transilluminator after labeled by ethidium bromide. Fragments containing 177 and 69 bp appear when there is a recognition side of HinfI RE enzyme in homozygous polymorphic type genotype. However, there is not any RE recognition side for the enzyme in homozygote wild type genotype so, only a fragment of 246 bp appears (Figure 1).

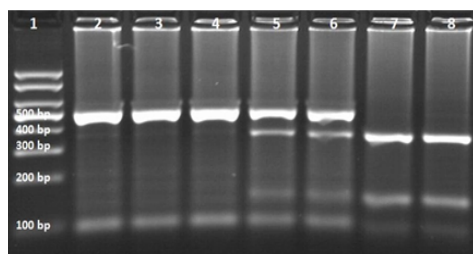


Figure 1. Imaging of Genotypes of MTHFR C677T Polymorphism on Agarose gel of 2.5%. 1-100 base pair (bp) DNA ladder; 2-PCR product (246 bp); 3,4-homozygous wild type genotype (CC) including a fragment of 246 bp; 5,6-heterozygous genotype (CT) including fragments of 246, 177 and 69 bp; 7,8-homozygous polymorphic type genotype (TT) including fragments of 177 and 69 bp

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences 15.0 programme (SPSS inc., Chicago, IL, USA). Mean age and body mass index (BMI) were calculated by Independent T test. The chi-square test (χ^2) was used for evaluate to relationship among gender differences, smoking habit, alcohol consumption, and allele-genotype frequencies. The odds ratios (OR) and 95% confidence interval (CI) were also calculated using χ^2 test. P values of <0.05 were considered statistically significant.

Results

Total 200 subjects including 100 patients with LC and 100 healthy controls were analyzed in this study. Distributions of age and gender were similar between two groups. The characteristics of the subjects were shown in Table 1. The frequencies of smoking habit and family history were significantly higher in the patients compared with the controls ($p<0.001$ for each analysis). BMI was found to be significantly lower in the patients than the controls ($p=0.01$). The thirteen times risk rate for development of LC was observed in smokers compared with non-smokers between patient and control groups (OR:9.65, 95%CI: 2.79-33.36; $p:0.0001$). The risk ratio for development of LC was nine times in individuals with cancer history in their family than those without cancer history in current study. Furthermore, BMI was significantly lower in patients with LC (24.8±4) than controls (OR:13.5, 95%CI:6.27-29.04, $p:0.0001$).

The genotypes distributions of MTHFR C677T polymorphism among groups were fitted Hardy-Weinberg equilibrium. Frequencies of genotypes and alleles of patients with LC and controls were shown in Table 2. We found no a significantly difference between the groups ($\chi^2:0.53$, $p=0.76$). The distributions of genotypes did not differ neither in patients with recurrence of cancer ($\chi^2:0.97$, $p:0.61$) nor in patients with metastasis ($\chi^2:3.56$, $p:0.16$). In addition, there was no any association between genotypes

Table 1. The Characteristics of Patients with Lung Cancer and Controls

	Patients N (%)	Controls N (%)	P value
Mean age ±SD (Year)	60.37±9.25 (38-81)	59.19±9.99 (35-80)	0.31
BMI ±SD (Kg/m ²)	24.8±4.5 (18-35)	26.2±3.7 (16-40)	0.01*
Gender(Male/female)	91/9 (91/9)	91/9 (91/9)	
Family history (No/Yes)	77/23 (77/23)	97/3 (97/3)	0.001*
Smoking habit			
(Non-smoker/Smoker)	10/90 (10/90)	60/40 (60/40)	0.001*
Histological type of patients			
SCLC	9 (9%)		
NSCLC	91 (91%)		
Adenocarcinoma	25 (27.5%)		
Squamous carcinoma	41 (45%)		
Others	25 (27.5%)		
Recurrence of cancer			
Yes	92 (92%)		
No	8 (8%)		
Metastases			
Yes	62 (62%)		
No	38 (38%)		

* $P<0.05$: statistically significant; §BMI: Body mass index; SD: Standard derivation; SCLC: small- cell carcinoma, NSCLC: non-small-cell carcinoma

Table 2. Distributions of Genotype and Allele among Patient Groups and Controls and Odds Ratios

		Patients N (%)	Controls N (%)	Crude		Adjusted*	
				OR (95%CI)	P Value	OR (95%CI)	P Value
Genotype	CC	55 (55)	51 (51)	Reference		Reference	
	CT	38 (38)	43 (43)	0.81 (0.45-1.46)	0.55	0.33 (0.07-1.60)	0.17
	TT	7 (7)	6 (6)	1.08 (0.34-3.43)	1	0.44 (0.09-2.07)	0.3
	CT+TT	45 (45)	49 (49)	0.85 (0.48-1.48)	0.67	1.87 (0.91-3.83)	0.08
Alleles	C	148 (74)	145 (72.5)	Reference			
	T	52 (26)	55 (27.5)	0.92 (0.59-1.44)	0.82		
				OR (95%CI)	P Value	OR (95%CI)	P Value
Histological types of LC	CC	6 (66.7)	49 (53.8)	Reference		Reference	
	CT	3 (33.3)	35 (38.5)	0.70 (0.16-2.99)	0.73	0.10 (0.01-3.23)	0.04
	TT	0 (0)	7 (7.7)				
	CT+TT	3 (33.3)	42 (46.2)	0.58 (0.13-2.47)	0.5	0.17 (0.01-0.99)	0.05
Alleles	C	15 (83.3)	133 (73.1)	Reference			
	T	3 (16.7)	49 (26.9)	0.54 (0.15-1.95)	0.41		

*Adjusted for smoking habit and history of family and BMI

of MTHFR C677T polymorphism and histological type of LC (χ^2 :0.99, $p=0.60$), (Table 2). TT genotype of the polymorphism was not detected in patients with SCLC in the study. Besides, there was not statistically significant difference for genotypes distributions of MTHFR C677T polymorphism neither early stages nor advanced stages in NSCLC and SCLC ($p>0.05$).

Discussion

Until now, the association between MTHFR C677T polymorphism and various cancers including breast cancer (Papandreou et al., 2012), gastric cancer (Gao et al., 2013), colorectal cancer (Levine et al., 2010), prostate cancer (Wu et al., 2010), hepatocellular cancer (Kwak et al., 2008) has been analyzed in several researches (Izmirli, 2013). Furthermore, several studies examining the role of the MTHFR C677T polymorphism in lung cancer susceptibility have been performed, but the results of these studies are contradictory: a significant correlation between MTHFR C677T polymorphism and LC was reported in some of these studies (Siemianowicz et al., 2003; Gemignani et al., 2007; Hung et al., 2007; Matakidou et al., 2007; Liu et al., 2008; Boccia et al., 2009; Cui et al., 2011; Kiyohara et al., 2011; Cheng et al., 2012; Liu et al., 2013), while the same correlation was not defined among them in other studies (Shen et al., 2001; Jeng et al., 2003; Mao et al., 2008; Liu et al., 2009; Zhang et al., 2012). Moreover, the association of MTHFR 677TT genotype and the subtypes of LC including NSCLC and SCLC have also been investigated in few studies. Shen et al. and Suzuki et al. could not show the association of MTHFR 677TT genotype and risk for subtypes of LC (Shen et al., 2003; Suzuki et al., 2007). However, some authors reported that the genetic models of MTHFR C677T polymorphism showed a significant association with an increasing risk of NSCLC (Arslan et al., 2011; Hou et al., 2012; Zhu et al., 2013). Different ethnic group, sample size (particularly small groups), diet, habits, environmental and genetic factors, gene-gene and gene-environment interactions and methodologies might be responsible for the discrepancy.

In this study, we evaluated the association between

MTHFR C677T polymorphism and LC and the subtypes of LC. This study is second research which was performed in the Central Anatolia Region of Turkey (Sivas). Aslan et al. performed to the first study with 64 cases and 61 controls. These authors reported that, there will be new studies with higher numbers of patients for confirming their results for Central Anatolia Region of Turkey. We suggested that the higher numbers of sample size may be affect the conclusion of the study. So, in current study, we investigated 100 patients with LC who were followed at the oncology hospital in Sivas in 2013 that the count identifies approximately number of annual total cases with LC. When Mao et al., investigated the effect of MTHFR C677T polymorphism on the lung cancer in a meta-analysis including 5,111 cases and 6,415 controls; they found no evidence a considerable role of the polymorphism in carcinogenesis of lung cancer (Mao et al., 2008).

As the research, we did not found any significant association between MTHFR C677T polymorphism and LC, either. Besides, in the present study, the high risk ratio of T allele for LC was not detected among the case and control groups. In addition, we did not find any association between histological type of LC including NSCLC and SCLC, and MTHFR 677TT genotype. We did not estimate to effect of TT genotype on SCLC because of MTHFR 677TT genotype might not be detected in this group. Contrary, an association of MTHFR 677TT genotype for LC was defined in other meta-analysis has been performed (Boccia et al., 2009). The OR of lung cancer was found 1.22 (95%CI: 0.95-1.55) for TT genotype of MTHFR C677T polymorphism by Boccia and colleagues that they analyzed 2076 cases and 4834 controls in the study. Furthermore, Aslan and colleagues (Aslan et al., 2011) found remarkable risk ratios of MTHFR C677T polymorphism for patients with TT genotype in development of LC and in NSCLC that these ratios were not statistically significant. The frequency of TT genotype was higher in patients than controls (11% vs 5%, respectively) in their study, whereas the rate was 7% in the patient group and 6% in the controls in current study. Our findings which related with number

of LC subtypes were similar to the results of Heijmans et al. (2003), and Hung et al. (2007) rather than results of Aslan's study. Besides, we suggest that a remarkable finding in our study will be reason of the discrepancy. The subtypes of NSCLC were not been identified in the most of studies. We found that the numbers of patients with squamous type LC (41 patients) were higher than numbers of patients with adenocarcinoma type LC (25 patients). Despite our finding, it has been declared that adenocarcinoma has surpassed squamous cell carcinoma in most of Asian countries and western countries. In addition, it suggest that this increasing of adenocarcinoma incidence appear to be attributable partially to the changed smoking pattern and elevating incidence of LC among females and non-smokers (Janssen-Heijnen et al., 2003; Wakelee et al., 2007).

Gender and smoking are important risk factors for LC. Dominant (TT homozygote and CT heterozygote vs CC homozygote) and allelic contrast (T allele vs C allele) models of MTHFR C677T polymorphism have been observed a considerable protective value on predisposed to LC in smokers rather non-smokers (Zhu et al., 2013). Besides, it has been found that the higher risk value in males for recessive model (TT homozygote vs CC homozygote and CT heterozygote) has been obtained in spite of TT homozygote and T allelic contrast had a protective value in females in the study (Zhu et al., 2013). The similar findings have been found by Zhang and colleagues (Zhang et al., 2012). However, these researchers decelerated that more predominant effect of smoking habit on LC in male than female was not affected by either MTHFR polymorphism nor folat intake (Liu et al., 2013). In accordance with this study, male ratio (91%) was quite high than female ratio (9%), in our study. Furthermore, smoking habit ratio was higher in males (90%) rather in females (10%), was detected. Although, smoking habit between patients and controls shown a significant difference but we did not observe any correlation of C677T polymorphism neither gender nor smoking habit. Suzuki et al. was not defining significant association between MTHFR TT genotype and LC risk in smoking individuals, either (Suzuki et al., 2007). We suggested that the effect of smoking habit on LC risk is independent from genotypes of MTHFR C677T polymorphism.

The results of our study suggest that there was not any correlation between MTHFR C677T polymorphism and LC in our Turkish population. There is a limitation of our study. The folate intake of the case and control groups was no questioned. Despite the limitation, given these results, the conclusion should be interpreted with caution owing to the heterogeneity. Further researches including larger size populations, and more comprehensive findings (gene-gene and gene-environment interactions and other risk factors for lung cancer) are necessary to detect whether there is significant association of MTHFR C677T polymorphism in LC in Turkish population.

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