RESEARCH COMMUNICATION

MTHFR C667T Polymorphism Association with Lung Cancer Risk in Henan Province: A Case-control Study

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Abstract

The current study was performed to assess any association between the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and risk of lung cancer in Henan province. This case-control study involved94 patients with newly histological confirmed lung cancer and 78 healthy controls. Genotyping was achieved with peripheral blood lymphocytes DNA and association of the polymorphism with risk of lung cancer was estimated by unconditional logistic regression analysis. The frequencies of the MTHFR 667TT genotype were 37.2% in cases compared with 23.1% in controls ($\chi 2 = 4.008$, P = 0.045). Individuals with the 667CC/CT genotype displayed a significantly reduced risk of lung cancer compared with those with the TT genotypes [adjusted odds ratio (OR), 0.506; 95% confidence interval (95% CI), 0.258 – 0.991]. The C667T polymorphism might have a significant effect on the occurrence of lung cancer in Henan province.

Keywords: MTHFR - mononucleotide polymorphism - lung cancer - susceptibility

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Introduction

Lung cancer is one of the most common malignancies and is the leading cause of cancer-related death worldwide (Jemal et al., 2010). Although epidemiologic studies have proved multiple risk factors such as age, gender, smoking, environmental pollution related to the occurrence of lung cancer, there are significant variations between individuals when exposed to same risk factors, for instance cigarette smoking, which is the primary risk factor for lung cancer, induces only 10-20% of lifetime smokers to develop lung cancer (Shields, 2002), this observation suggests that other factors, such as genetic susceptibility resulted from polymorphisms of genes involving chemical metabolism, DNA repair, cell cycle regulation and immunity may influence risk of lung cancer (Dong et al., 2008).

Single nucleotide polymorphisms (SNPs), which represents an alternate nucleotide in a given and defined genetic location and occurs on average every 100 to 1000 base pairs in vertebrates, is used to establish differences between individuals (Gill, 2001) and is a useful markers for propensity to disease (Amos et al., 2008), disease states (Poehlmann et al., 2007), as well as evidence of the genetic basis of adaptation (Hoekstra et al., 2006). Methylene tetrahydrofolate reductase (MTHFR) is a key enzyme in metabolism of folate and nucleotides to maintain DNA stability and prevent cancers through catalyzing irreversible conversion of 5, 10-methylenetetrahydrofolate (5, 10-methyleneTHF) to 5-methyl tetrahydrofolate (5-methyl THF) (Botto and Yang, 2000). MTHFR C677T, which transits C to T transition at nucleotide 677 and causes an alanine-to-valine substitution in the MTHFR protein at amino-acid 222, is the most extensively investigated MTHFR polymorphism besides A1298C and G1793A, with impaired enzyme activity and heat stability (Guenther et al., 1999). To date, growing evidences have proved that MTHFR C677T polymorphism are associated with bladder cancer, pancreatic cancer, malignant lymphoma (Matsuo et al., 2001; Lin et al., 2004; Li et al., 2005). However, as for the role of the MTHFR C677T polymorphism in lung cancer susceptibility, no consistent conclusion can be drawn from the limited information available including large sample and meta-analysis.

Considering MTHFR C677T polymorphism is affected by ethnic groups, environment, and its biological effects significantly disturbed by nutritional conditions such as different folate in-taken, in this study, the genotypic distributions of MTHFR C677T among lung cancer patients and healthy people from north of Henan province with same ethnic, environment and similar diet habit were detected respectively in order to explore the possible association between MTHFR C677T polymorphism and lung cancer susceptibility.

Materials and Methods

Samples

The study population consisted of 94 patients with newly histologically diagnosed lung cancer and 78 population-based controls. All subjects were recruited at

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1st affiliated hospitals of Zhengzhou University or Institute of Clinical Medical Research of Henan Universities. All of them were native and permanent resident of north Henan province with same ethnic of Han, environment and similar diet habits especially in vegetables and fruits in-taken. All enrolled patients didn't receive any radiation or chemical therapy treatment before operation and hadn't secondary or recurrent tumors. 78 healthy people were randomly selected as the controls. Participants who smoked no less than one cigarette per day or those who smoked for more than half a year was categorized as "smokers," and the rest were as "Non-smokers." At the time of their peripheral blood collections, all subjects provided their informed consent to participate in this study. Their basic data were collected using questionnaire.

Detection and verification of MTHFR genotypes

DNA was collected from the samples of peripheral blood lymphocytes. The sequencing primers of MTHFR were designed and amplified as Jin's (Jin et al., 2007), synthesized by the Shanghai Sangon Company (Shanghai, China). After electrophoresis detection, purified PCR products of 20 cases successfully digested by Hinf I and randomly selected were recycled and connected to Shanghai Sangon Company to verify the sequence of MTHFR genotypes, then the sequence was read in Software chromas 2.23 and alignment in BLAST (http://blast.ncbi.nlm.nih.gov/blast.cgi).

Statistical analysis

The statistical significance of differences between cases and controls were estimated by χ^2 test. Adjusted odds ratios (OR) were calculated with a logistic regression model that controlled for gender and age are given with 95% confidence intervals (CI). Subjects with the mutation-type genotypes (MTHFR 677TT) were considered to be at baseline risk. The expected frequency of control genotypes was checked by the Hardy-Weinberg equilibrium test through on-line testing software (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). All analyses were performed using the Statistical Package for the Social Sciences software (ver. 16.0; SPSS, Chicago, IL, USA). P values ≤ 0.05 were considered to be statistically significant.

Table 1. General Characteristics of Subjects

Characteristics	Cases	group
No.	94	78
Age (mean±SD)	60.23±9.087	62.05±8.105
Gender		
Male	73	59
Female	21	19
Smoking status*		
Smoker	53	23
Non-Smokers	41	55
Histological Type		
Adenocarcinoma	31	
Squmous cell carcinoma	47	
Small cell lung cancer	3	
Others	13	

*p<0.01

Results

General characteristics of subjects

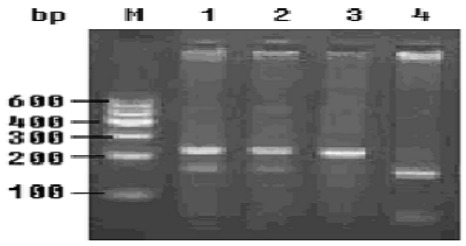
The characteristics of the study population are presented in (Table1) in total, 94 cases and 78 controls were included in these analyses. The 94 lung cancer cases consisted of 31 adeno-carcinomas, 47 squamous-cell carcinomas, 3 small cell carcinomas, and 13 other types, including 4 large cell cancers, 5 mixed types and 4 non-histological type. The ages ranged from 36 to 81 years old in cases, and 35 to80 years old in controls. There were no significant differences in mean age and gender between two groups. While the proportion of smokers in lung cancer cases was higher than in the controls (χ^2=12.139, P <0.001)

Genotypic distribution of MTHFR C667T

As shown in Figure 1 and verified in Figure 2, the amplified MTHFR DNA fragments of 246bp produced three genotypes after being digested with Hinf1 restriction enzyme: wide type (CC, containing fragments of 246bp), heterozygote (CT, containing fragments both of 246bp and 174bp) and homozygote mutation (TT, containing fragments of 174bp). The genotypic frequency in controls satisfied standard of Hardy-Weinberg equilibrium (χ^2 < 0.001, P = 0.990). The difference in genotypic frequency between the cases and controls had no statistical significance (χ^2 = 5.040, P = 0.081) (Table 2).

MTHFR C667T genotypes and the risk of lung cancer

The genotype distributions for MTHFR C677T gene polymorphisms and their ORs and 95% CIs in lung cancer



M : 100bp ladder; 1 and 2 :CT; 3:CC; 4: TT;

Figure 1. Electrophoresis Chart of PCR Products Containing Site C677T of MTHFR Digested by Hinf I

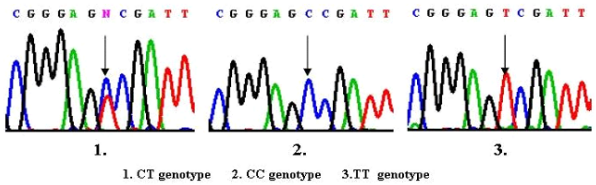


Figure 2. The Sequencing of MTHFR C677T Site Digested by Hinf I (Points to SNP)

Table 2. Distribution of MTHFR C667T Genotypic Frequencies (n%)

MTHFR C667T	Cases (%)	Controls (%)
C/C	26(27.7%)	21(26.9%)
C/T	33(35.1%)	39(50%)
T/T	35(37.2%)	18(23.1%)

Table 3. MTHFR C667T Genotypes and the Risk of Lung Cancer

MTHFR C667T	Cases	Controls	χ^2	OR*	95%CI	P*
C/C	26	21	1.2	0.637	0.284-1.429	0.273
C/T	33	39	5.02	0.435	0.209-0.906	0.025
C/T +C/C	59	60	4.01	0.506	0.258-0.991	0.045
T/T	35	18		1**		

*Adjusted for age, gender; **Reference

was shown in Table 3. Results showed that proportion of TT genotypic in the cases was higher than that in the controls ($\chi^2 = 4.008$, P = 0.045). Regarded MTHFR 677TT genotype as the reference group, 677 CT and 677 CT/CC had a 0.435-fold and 0.506-fold reduced risk of lung cancer, respectively (OR = 0.435, 95% CI = 0.209 – 0.906, P = 0.025; OR = 0.506, 95% CI = 0.258 – 0.991, P = 0.045), though the protection effect of 677 CC did not reach statistical significance (OR = 0.637, 95% CI = 0.284 - 1.429, P = 0.273).

Discussion

This hospital-based case-control study showed a significant association between the MTHFR C677T polymorphism and risk of lung cancer in human from north of Henan province. Individuals carrying with heterozygous CT genotype or with the CC wild-type genotype had a 0.506-fold reduced risk of developing lung cancer compared with individuals with the homozygous TT genotype.

MTHFR is the critical juncture in folate metabolism by catalyzing the irreversible conversion of 5, 10-methylene THF to 5-methyl THF, which is not only involved in so-called 'one-carbon metabolism' to facilitate de novo deoxynucleoside triphosphate synthesis, but also provides methyl groups required for intracellular methylation reactions, thereby enhancing DNA synthesis and repair ability and preventing cancers. Although its amino acide sequence is proved highly conserved, there are several polymorphisms of the MTHFR gene identified. MTHFR C677T, which transits C to T transition at nucleotide 677, causes an alanine-to-valine substitution in the MTHFR protein at amino-acid 222 and results in reduction in the enzyme activity by 70% in homozygous TT genotype and 35% in heterozygous CT genotype, compared with normal CC genotype (Frosst et al., 1995), with subsequent reduced 5-methyl THF and impaired DNA synthesis and repair ability (Friso and Choi, 2005), which finally proved to increase the risk of neural defects, cardiovascular disease and tumors such as bladder cancer, pancreatic cancer, malignant lymphoma (Matsuo et al., 2001; Lin et al., 2004; Li et al., 2005).

Lung cancer is one of the most common malignancies and is the leading cause of cancer-related death worldwide (Jemal et al., 2010). However, results of several studies examining the role of the MTHFR C677T polymorphism in lung cancer susceptibility have been inconsistent. Arslan et al., (2011) in Turkey, Siemianowicz et al., (2003) in Poland, Hung et al., (2007) in Central Europe, Shen et al., (2005) in China and a recent meta-analysis by Boccia et al., (2009) in Italy based on 10 case-control studies showed that individuals with MTHFR TT genotype had an increased risk of lung cancer versus those with the wild-type homozygous variant, while others studies such as Suzuki et al., (2007) in Japan, Cui et al., (2011) in Korea and another -meta-analysis by Mao et al., (2008) in china based on 8 case-control studies suggested no evidence for a major role of the MTHFR C677T polymorphisms in carcinogenesis of lung cancer, regardless Liu et al., (2009) and Jeng et al., (2003) in Taiwan and Shi et al., (2005) in Houston, TX showed that the MTHFR 677 TT genotype was associated with a decreased risk of lung cancer.

Although different sample size and methodologies might be responsible for this discrepancy, the effect-ion that various ethnic groups, environment, diet habit on MTHFR C677T polymorphisms might be also contributed to this inconsistent results. Pepe et al., found the mutation rate of MTHFR C677T was lower among Black in Africa than European and Asian population, and was also different among Black located various environment (Pepe et al., 1998), Yu et al., detected that the T allele frequency differences are apparent among different ethnic groups in China (1998). In addition, it was reported biological effects of MTHFR C677T polymorphisms was significantly disturbed by nutritional conditions such as different folate in-taken (Li et al., 2005; Eaton et al., 2005), Considering these, native and permanent resident of north Henan province with same ethnic of Han, environment and similar diet habits especially in vegetables and fruits in-taken were enrolled and found that TT genotype is a risk factor of lung cancer. The results in our study showed that there was a significant difference in the distribution of TT genotype between the case and control groups, Individuals carrying with heterozygous CT genotype or with the CC wild-type genotype had a 0.506-fold reduced risk of developing lung cancer compared with individuals with the homozygous TT genotype, which still must be further confirmed with large sample.

In summary, TT genotype of MTHFR C677T might be a susceptible factor of lung cancer occurrence. Thus, the prevention of TT among susceptible population will be one of the important ways to prevent and control lung cancer.

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