CANCER GENOMICS & PROTEOMICS *6*: 325-330 (2009)

Interaction of Methylenetetrahydrofolate Reductase Genotype and Smoking Habit in Taiwanese Lung Cancer Patients

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Abstract. *The aim of this study was to evaluate the association and interaction of genotypic polymorphisms in methylenetetrahydrofolate reductase (MTHFR) and environmental factors with lung cancer in Taiwan. Two well-known polymorphic variants of MTHFR, C677T (rs1801133) and A1298C (rs1801131), were analyzed in association with lung cancer susceptibility, and discussed their joint effects with individual habits on lung cancer risk. Patients and Methods: In total, 358 patients with lung cancer and 716 healthy controls recruited from the China Medical Hospital in central Taiwan were genotyped. Results: The MTHFR C677T genotype, but not the A1298C, was differently distributed between the lung cancer and control groups. The T allele of MTHFR C677T was significantly more frequently found in controls than in cancer patients. As for A1298C polymorphism, there was no difference in distribution between the lung cancer and control groups. Gene interactions with smoking were significant for MTHFR C677T polymorphism. The MTHFR C677T CT and TT genotypes in association with smoking conferred a decreased risk of 0.706 (95% confidence interval=0.531-0.939) for lung cancer. Conclusion: Our results provide the first evidence that the C allele of MTHFR C677T may be associated with the development of lung cancer and may be a novel useful marker for primary prevention and anticancer intervention.*

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Key Words: MTHFR, polymorphism, lung cancer, carcinogenesis.

All over the world, lung cancer has become one of the most common malignancies (1, 2). In Taiwan, lung cancer is important for its high incidence, high mortality, and low 5-year survival rate, especially in female adenocarcinoma cases (3). Among the well-known lifestyle-related causes of lung cancer, smoking is considered to be the most important (4-6).

In recent years, environmental and genomic susceptibilities and interactions among them were used in evaluation of cancer risk. Primary candidates for gene-environment interaction studies are those encoding enzymes related to the metabolism of established carcinogens. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism and catalyzes 5, 10-methylene-tetrahydrofolate to 5-methyltetra-hydrofolate. The importance of MTHFR in cancer susceptibility arises from its involvement in two pathways of folate metabolism: one leads to numerous methylation processes that are dependent on *S*-adenosyl-methionine (SAM), while the other, *via* thymidylate synthesis, contributes to DNA replication and cell division. Reduced activity of MTHFR may decrease the methylation of homocysteine to methionine and in turn the level of SAM, resulting in DNA hypomethylation. On the other hand, a reduced level of MTHFR substrate, required for thymidylate synthesis could lead to uracil misincorporation into DNA, diminished DNA repair and increased frequency of chromosomal breaks and damage. Malignancies that are derived from rapidly proliferating tissues, which have a higher requirement for DNA synthesis, should be more susceptible to folate deficiency and resultant DNA damage. The DNA variants causing reduced MTHFR activity were found to be associated with a reduced risk of leukemia, lymphoma and colorectal carcinoma. The mechanism proposed to explain these associations was the shunt of folate metabolism *versus* thymidine and purine synthesis, which would slow the incorporation of uracil into DNA and protect the cells against carcinogenesis (7).

Previous investigations of *MTHFR* genetic variations focused on the catalytic domain and the two polymorphisms

Table I. *The primer sequences, polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) conditions for MTHFR gene polymorphisms.*

Polymorphism (location)	Primers sequences (5'->3')	Restriction enzyme	SNP sequence	DNA fragment size (bp)
C677T	F: TGA AGG AGA AGG TGT CTG CGG GA	*Hinf I*	C	198
(rs1801133)	R: AGG ACG GTG CGG TGA GAG TG		T	175 + 23
A1298C	F: GGGAGGAGCTGACCAGTGCAG	*Fnu4H I*	C	138
(rs1801131)	R: GGGGTCAGGCCAGGGGCAG		A	119 + 19

*F and R indicate forward and reverse primers, respectively.

C677T and A1298C, which slightly change enzymatic activity. In the case of C677T polymorphism, the cytosine base at position number 677 changes to a thymidine base, which in turn affects the amino acid sequence at position number 222 (alanine→valine). The *MTHFR* C677T variants are *MTHFR* 677CC, which is the wild type (most common), *MTHFR* 677CT and *MTHFR* 677TT. The MTHFR enzyme resulting from the polymorphism becomes thermo-labile, causing a loss of its activity with increased temperature. The modified protein loses its cofactor flavin adenine dinucleotide (FAD) more quickly and has a lower stability. The mutation effect can be suppressed by addition of folate, which causes a higher FAD affinity and an increase in MTHFR stability. The *MTHFR* A1298C polymorphism is localized in the coding regulatory region domain (8). Studies investigating the *MTHFR* A1298C variant have found positive associations with colorectal cancer (9), breast cancer (10), acute lymphocytic leukemia (11) and childhood leukemia (12), but none with lung cancer suscepitibility.

In 2003, the association between single nucleotide polymorphism (SNPs) of *MTHFR* and lung cancer susceptibility was firstly examined in a Taiwanese population, indicating the C677T is not associated with lung cancer risk (13). However, the sample size was rather small (control/case=232/59), and only one SNP was investigated in the study. In the present work, we analyzed the genetic polymorphisms of both *MTHFR* C677T and A1298C in a more representative population (control/case=716/358), and investigated the interaction of *MTHFR* genotypes and smoking habits in a Taiwanese population.

Patients and Methods

Study population and sample collection. Three hundred and fifty-eight cancer patients diagnosed with lung cancer were recruited at the outpatient clinics of general surgery between 2005-2008 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The clinical characteristics of patients including histological details were all graded and defined by expert surgeons. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. Twice as many non-lung cancer healthy volunteers as controls were selected by matching for age, gender and habits after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. Both groups completed a short questionnaire which included habits. Our study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants.

Genotyping assays. Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous studies (14-22). The polymerase chain reaction (PCR) cycling conditions were: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s, and a final extension at 72˚C for 10 min. Pairs of PCR primer sequences and restriction enzyme for each DNA product are all listed in Table I.

Statistical analyses. Only those with both genotypic and clinical data (control/case=716/358) were selected for final analysis. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *MTHFR* SNPs in the controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's χ^2 test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the genotypes between cases and controls. Data were recognized as significant when the statistical *p*-value was less than 0.05.

Results

The frequency distributions of selected characteristics of 358 lung cancer patients and 716 controls are shown in Table II. These characteristics of patients and controls are all well matched. None of the differences between both groups were statistically significant ($p>0.05$) (Table II).

The frequencies of the genotypes for the *MTHFR* C677T and A1298C in controls and lung cancer patients are shown in Table III. The genotype distribution of the genetic polymorphisms of *MTHFR* C677T was significantly different between lung cancer and control groups ($p=0.0002$), while that for A1298C polymorphisms were not significant ($p>0.05$) (Table III). The frequencies of the alleles for *MTHFR* C677T and A1298C in controls and lung cancer patients are shown in

Table II. *Characteristics of lung cancer patients and controls*.

Characteristic	Controls (n=716)			Patients (n=358)			*P*-value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			64.8 (6.8)			64.0 (6.9)	0.58
Gender							
Male	488	68.1%		254	70.9%		0.36
Female	228	31.9%		104	29.1%		
Habit							
Cigarette smokers	563	78.6%		293	81.8%		0.23
Non-smokers	153	21.4%		65	18.2%		

[a]Based on χ^2 test.

Table III. *Distribution of MTHFR genotypes among lung cancer patient and control groups*.

Genotype	Controls	%	Patients	%	*P*-value[a]
C677T rs1801133					0.0002
CC	362	50.6%	205	63.7%	
CT	291	40.6%	124	30.2%	
TT	63	8.8%	29	6.1%	
A1298C rs1801131					0.6855
AA	467	65.2%	228	63.7%	
AC	226	31.6%	115	32.1%	
CC	23	3.2%	15	4.2%	

[a]Based on χ^2 test.

Table IV. *MTHFR allelic frequencies among the lung cancer patient and control groups*.

Allele	Controls	%	Patients	%	*P*-value[a]
C677T rs1801133					0.0742
Allele C	1015	70.9%	534	74.6%	
Allele T	417	29.1%	182	25.4%	
A1298C rs1801131					0.4875
Allele A	1160	81.0%	571	79.8%	
Allele C	272	19.0%	145	20.2%	

[a]Based on χ^2 test.

Table IV. The C allele of the *MTHFR* C677T polymorphism seems to be associated with lung cancer while not significant (p=0.0742). The conclusion deduced from the data in Tables III and IV is that *MTHFR* C677T T allele seems to be associated with lower risk for lung cancer in Taiwan.

Since smoking is the predominant risk factor for lung cancer, the interaction between *MTHFR* genotype and individual smoking habits was also analyzed by stratified individual smoking status (Table V). We noticed that subjects with the heterozygous CT for *MTHFR* C677T had lower risks of lung cancer in smoking group, but not in the case of non-smoking group. There was an interaction between smoking status and *MTHFR* genotypes in the lung cancer susceptibility.

Discussion

In order to elucidate the role of *MTHFR* and to find potential biomarkers of lung cancer, in this study, we selected two SNPs of the *MTHFR* gene and investigated their associations with the susceptibility for lung cancer in a population of central Taiwan. We found that the T variant genotypes of *MTHFR* C677T were significantly associated with a lower susceptibility for lung cancer (Tables III and IV). These data are inconsistent with those finding the T allele to be of a higher risk (23-25), or of it having no association with lung cancer (13, 26-31). This may be caused by differences in ethnicity; moreover, our sample size was larger than that of Jeng *et al*. and more representative of Taiwan (13). Thus, the effects of the *MTHFR* C677T polymorphism on carcinogenesis are complex, exerting either an adverse effect on DNA methylation or an advantageous influence on nucleotide synthesis in determining cancer risk.

We have further analyzed the association between C677T genotype and lung cancer risk in patients and controls who have a cigarette smoking habit. Interestingly, the interaction between *MTHFR* C677T and cigarette smoking habit is clear: smokers with the CT genotype have a 0.69-fold lower risk of lung cancer than those smokers with CC genotype, which is not the case in the non-smoking people (Table V). We propose that the C allele of C677T may affect MTHFR activity, slightly influencing its normal function. As individuals with the C allele(s) get older, the alteration towards carcinogenesis may accumulate *via* the decreasing function of MTHFR. Cigarette smoking, a well-known cause of DNA damage, will release many DNA damage

Table V. *Distribution of MTHFR C677T genotype and lung cancer after stratification by smoking habit.*

SNP/Genotype	Overall			Never smokers			Ever smokers		
	Controls N (%)	Cases N (%)	Adjusted[a] OR (95% CI)[c]	Controls N (%)	Cases N (%)	Adjusted[b] OR (95% CI)[c]	Controls N (%)	Cases N (%)	Adjusted[b] OR (95% CI)[c]
C667T (rs1801133)									
CC	362 (50.6)	205 (63.7)	1.00 (Ref.[d])	80 (35.74)	33 (29.27)	1.00 (Ref.[d])	282 (33.33)	172 (35.07)	1.00 (Ref.[d])
CT	291 (40.6)	124 (30.2)	0.73 (0.54-0.96)	59 (47.30)	26 (48.98)	1.07 (0.58-1.97)	232 (49.84)	98 (49.50)	0.69 (0.51-0.92)
TT	63 (8.8)	29 (6.1)	0.80 (0.52-1.32)	14 (10.97)	6 (21.75)	1.04 (0.37-2.94)	49 (16.83)	23 (15.44)	0.77 (0.45-1.41)

[a]Adjusted for age, gender and smoking (pack-years); [b]Adjusted for age and gender; [c]OR, odds ratio; CI, confidence interval; [d]Ref., reference.

inducers into the respiratory system and cause DNA damage to cells. Therefore, in people who have a risky carriers of the genetic variant, such as C allele of C677T, and who also have a smoking habit, the joint effect of these factors may synergistically increase their lung cancer susceptibility.

To sum up, this is the first study which focuses on the SNPs of *MTHFR* and their joint effects with smoking habit on lung cancer risk in Taiwan, and the presence of the C allele of C677T was associated with a higher risk of lung cancer. The C allele of *MTHFR* C677T may be a useful marker in lung oncology for anticancer application, and early cancer detection.

Acknowledgements

We thank Yung-Shun Kuo, Hua-Shiang Chen, Yu-Shan Huang, Wen-Shin Chang, Hsiu-Min Hsieh and the Tissue Bank at the China Medical University for their technical assistance. This study was supported by research grants from the Terry Fox Cancer Research Foundation, National Science Council (NSC 98-2320-B-039-010-MY3) and China Medical University and Hospital (DMR-99-014).

References

1 Chiu HF, Cheng MH, Tsai SS, Wu TN, Kuo HW and Yang CY: Outdoor air pollution and female lung cancer in Taiwan. Inhal Toxicol *18*: 1025-1031, 2006.

2 Parkin DM, Bray F, Ferlay J and Pisani P: Global cancer statistics, 2002. CA Cancer J Clin *55*: 74-108, 2005.

3 Jemal A, Siegel R, Ward E, Murray T, Xu J and Thun MJ: Cancer statistics, 2007. CA Cancer J Clin *57*: 43-66, 2007.

4 Yang L, Parkin DM, Ferlay J, Li L and Chen Y: Estimates of cancer incidence in China for 2000 and projections for 2005. Cancer Epidemiol Biomarkers Prev *14*: 243-250, 2005.

5 Yang L, Parkin DM, Li L and Chen Y: Time trends in cancer mortality in China: 1987-1999. Int J Cancer *106*: 771-783, 2003.

6 Zhang H and Cai B: The impact of tobacco on lung health in China. Respirology *8*: 17-21, 2003.

7 Krajinovic M, Lamothe S, Labuda D, Lemieux-Blanchard E, Theoret Y, Moghrabi A and Sinnett D: Role of *MTHFR* genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. Blood *103*: 252-257, 2004.

8 Homberger A, Linnebank M, Winter C, Willenbring H, Marquardt T, Harms E and Koch HG: Genomic structure and transcript variants of the human methylenetetrahydrofolate reductase gene. Eur J Hum Genet *8*: 725-729, 2000.

9 Chen J, Giovannucci E, Hankinson SE, Ma J, Willett WC, Spiegelman D, Kelsey KT and Hunter DJ: A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms, and risk of colorectal adenoma. Carcinogenesis *19*: 2129-2132, 1998.

10 Sharp L, Little J, Schofield AC, Pavlidou E, Cotton SC, Miedzybrodzka Z, Baird JO, Haites NE, Heys SD and Grubb DA: Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (*MTHFR*). Cancer Lett *181*: 65-71, 2002.

11 Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA and Morgan G: Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci USA *96*: 12810-12815, 1999.

12 Wiemels JL, Smith RN, Taylor GM, Eden OB, Alexander FE and Greaves MF: Methylenetetrahydrofolate reductase (*MTHFR*) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia. Proc Natl Acad Sci USA *98*: 4004-4009, 2001.

13 Jeng YL, Wu MH, Huang HB, Lin WY, You SL, Chu TY, Chen CJ and Sun CA: The methylenetetrahydrofolate reductase 677C→T polymorphism and lung cancer risk in a Chinese population. Anticancer Res *23*: 5149-5152, 2003.

14 Shrubsole MJ, Shu XO, Ruan ZX, Cai Q, Cai H, Niu Q, Gao YT and Zheng W: *MTHFR* genotypes and breast cancer survival after surgery and chemotherapy: a report from the Shanghai Breast Cancer Study. Breast Cancer Res Treat *91*: 73-79, 2005.

15 Chang CH, Chang CL, Tsai CW, Wu HC, Chiu CF, Wang RF, Liu CS, Lin CC and Bau DT: Significant association of an *XRCC4* single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 1777-1782, 2009.

16 Chang CH, Chiu CF, Liang SY, Wu HC, Chang CL, Tsai CW, Wang HC, Lee HZ and Bau DT: Significant association of *Ku80* single nucleotide polymorphisms with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 1275-1279, 2009.

17 Chiu CF, Tsai MH, Tseng HC, Wang CL, Wang CH, Wu CN, Lin CC and Bau DT: A novel single nucleotide polymorphism in *XRCC4* gene is associated with oral cancer susceptibility in Taiwanese patients. Oral Oncol *44*: 898-902, 2008.

18 Chiu CF, Wang CH, Wang CL, Lin CC, Hsu NY, Weng JR and Bau DT: A novel single nucleotide polymorphism in *XRCC4* gene is associated with gastric cancer susceptibility in Taiwan. Ann Surg Oncol *15*: 514-518, 2008.
19 Chiu CF, Wang HC, Wang CH, Wang CL, Lin CC, Shen CY, Chiang SY and Bau DT: A new single nucleotide polymorphism in *XRCC4* gene is associated with breast cancer susceptibility in Taiwanese patients. Anticancer Res *28*: 267-270, 2008.
20 Hsu CF, Tseng HC, Chiu CF, Liang SY, Tsai CW, Tsai MH and Bau DT: Association between DNA double strand break gene *Ku80* polymorphisms and oral cancer susceptibility. Oral Oncol *45*: 789-793, 2009.
21 Hsu NY, Wang HC, Wang CH, Chiu CF, Tseng HC, Liang SY, Tsai CW, Lin CC and Bau DT: Lung cancer susceptibility and genetic polymorphisms of Exo1 gene in Taiwan. Anticancer Res *29*: 725-730, 2009.
22 Yang MD, Hsu YM, Kuo YS, Chen HS, Chang CL, Wu CN, Chang CH, Liao YM, Wang HC, Wang MF and Bau DT: Significant association of *Ku80* single nucleotide polymorphisms with colorectal cancer susceptibility in Central Taiwan. Anticancer Res *29*: 2239-2242, 2009.
23 Hung RJ, Hashibe M, McKay J, Gaborieau V, Szeszenia-Dabrowska N, Zaridze D, Lissowska J, Rudnai P, Fabianova E, Mates I, Foretova L, Janout V, Bencko V, Chabrier A, Moullan N, Canzian F, Hall J, Boffetta P and Brennan P: Folate-related genes and the risk of tobacco-related cancers in Central Europe. Carcinogenesis *28*: 1334-1340, 2007.
24 Zhang XM, Miao XP, Tan W, Qu SN, Sun T, Zhou YF and Lin DX: Association between genetic polymorphisms in methylentetrahydrofolate reductase and risk of lung cancer. Zhongguo Yi Xue Ke Xue Yuan Xue Bao *27*: 700-703, 2005.
25 Siemianowicz K, Gminski J, Garczorz W, Slabiak N, Goss M, Machalski M and Magiera-Molendowska H: Methylenetetrahydrofolate reductase gene C677T and A1298C polymorphisms in patients with small cell and non-small cell lung cancer. Oncol Rep *10*: 1341-1344, 2003.
26 Heijmans BT, Boer JM, Suchiman HE, Cornelisse CJ, Westendorp RG, Kromhout D, Feskens EJ and Slagboom PE: A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer. Cancer Res *63*: 1249-1253, 2003.
27 Shen M, Rothman N, Berndt SI, He X, Yeager M, Welch R, Chanock S, Caporaso N and Lan Q: Polymorphisms in folate metabolic genes and lung cancer risk in Xuan Wei, China. Lung Cancer *49*: 299-309, 2005.
28 Shi Q, Zhang Z, Li G, Pillow PC, Hernandez LM, Spitz MR and Wei Q: Sex differences in risk of lung cancer associated with methylene-tetrahydrofolate reductase polymorphisms. Cancer Epidemiol Biomarkers Prev *14*: 1477-1484, 2005.
29 Suzuki T, Matsuo K, Hiraki A, Saito T, Sato S, Yatabe Y, Mitsudomi T, Hida T, Ueda R and Tajima K: Impact of one-carbon metabolism-related gene polymorphisms on risk of lung cancer in Japan: a case control study. Carcinogenesis *28*: 1718-1725, 2007.
30 Vineis P, Veglia F, Garte S, Malaveille C, Matullo G, Dunning A, Peluso M, Airoldi L, Overvad K, Raaschou-Nielsen O, Clavel-Chapelon F, Linseisen JP, Kaaks R, Boeing H, Trichopoulou A, Palli D, Crosignani P, Tumino R, Panico S, Bueno-De-Mesquita HB, Peeters PH, Lund E, Gonzalez CA, Martinez C, Dorronsoro M, Barricarte A, Navarro C, Quiros JR, Berglund G, Jarvholm B, Day NE, Key TJ, Saracci R, Riboli E and Autrup H: Genetic susceptibility according to three metabolic pathways in cancers of the lung and bladder and in myeloid leukemias in nonsmokers. Ann Oncol *18*: 1230-1242, 2007.
31 Shen H, Spitz MR, Wang LE, Hong WK and Wei Q: Polymorphisms of methylene-tetrahydrofolate reductase and risk of lung cancer: a case-control study. Cancer Epidemiol Biomarkers Prev *10*: 397-401, 2001.

Received September 30, 2009
Revised November 22, 2009
Accepted November 23, 2009