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.
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 susceptibility.

In 2003, the association between single nucleotide polymorphisms (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 $\chi^2$ 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 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
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.

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References


Liu et al: MTHFR Genotypes in Lung Cancer

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