Significant Association of Methylenetetrahydrofolate Reductase Single Nucleotide Polymorphisms with Prostate Cancer Susceptibility in Taiwan

HSI-CHIN WU[1,4,*], CHAO-HSIANG CHANG[1,4*], RU-YIN TSAI[1*], CHIH-HSUEH LIN[5], ROU-FEN WANG[1,2], CHIA-WEN TSAI[1,2], KUEN-BAO CHEN[1], CHUN-HSU YAO[3], CHANG-FANG CHIU[1], DA-TIAN BAU[1,2], and CHENG-CHIEH LIN[1,5]

[1]Terry Fox Cancer Research Laboratory, China Medical University Hospital, Taichung, Taiwan, ROC;
[2]Graduate Institute of Basic Medical Science, and [3]Department of Biomedical Imaging and Radiological Science, China Medical University, Taichung, Taiwan, ROC;
[4]Department of Urology, and [5]Department of Family Medicine, China Medical University Hospital, Taichung, Taiwan, ROC

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* These authors contributed equally to this work

Correspondence to: Da-Tian Bau and Cheng-Chieh Lin, Terry Fox Cancer Research Lab, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, P.R.C. Tel: +886 422053366 ext 3312, Fax: +886 422053366 ext 1511, e-mail: datian@mail.cmuh.org.tw; artbau1@yahoo.com.tw

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Abstract. Prostate cancer is the most common cause of cancer death in men and is a major health problem worldwide. Methylene tetrahydrofolate reductase (MTHFR) plays an important role for folate metabolism and is also an important source for DNA methylation and DNA synthesis (nucleotide synthesis). To assess the association and interaction of genotypic polymorphisms in *MTHFR* and lifestyle factors with prostate cancer in Taiwan, we investigated two well-know polymorphic variants of *MTHFR*, C677T (rs1801133) and A1298C (rs1801131), analyzed the association of specific genotypes with prostate cancer susceptibility, and discussed their joint effects with individual habits on prostate cancer risk. In total, 218 patients with prostate cancer and 436 healthy controls recruited from the China Medical Hospital in central Taiwan were genotyped investigating the association of these polymorphisms with prostate cancer susceptibility. We found the *MTHFR* C677T but not the A1298C genotype, was differently distributed between the prostate cancer and control groups. The T allele of *MTHFR* C677T conferred a significantly (P = 0.0011) decreased risk of prostate cancer. As for the A1298C polymorphism, there was no difference in distribution between the prostate 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 0.501 (95% confidence interval=0.344-0.731) for prostate cancer. Our results provide the

first evidence that the C allele of *MTHFR* C677T may be associated with the development of prostate cancer and may be a novel useful marker for primary prevention and anticancer intervention.

Prostate cancer is one of the most important diseases in men all over the world. In the men of the United States and Western Europe, prostate cancer is a leading cause of illness and death (1), while the incidence of prostate cancer widely varies in different races. According to the literature, Asians have the lowest incidence among the major races, and African—American men have the greatest incidence in the world (2). In Taiwan, although the incidence of prostate cancer is much lower compared with other countries, it still takes the seventh place in the top ten cancer causes of death for male Taiwanese (3). The number of patients and the death rate have also been increasing during the two decades (3), and prostate cancer has become a serious public threaten of Taiwanese mature males. In the literature, some risk factors have been confirmed as being associated with prostate cancer, including age, race, and a family history of prostate cancer (2). Additionally, smoking, together with diet, androgens, occupational chemicals, inflammation and obesity, have been considered as secondary risk factors (2).

In recent years, environmental and genomic susceptibilities and interactions among them have been used in evaluation of cancer risk. Primary candidates for gene—environment lifestyle interaction studies are those encoding enzymes related to the metabolism of established risk factors of carcinogenesis. Methylene tetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism, which catalyzes 5, 10-MTHF to 5-MTHF. 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, the reduced level of MTHFR substrate, 5,10-MTHF, 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 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 (3).

Previous investigations of *MTHFR* genetic variations focused on the catalytic domain and the two polymorphisms 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 wild type (most common), *MTHFR* 677CT heterozygous genotype and *MTHFR* 677TT homozygous genotype. The MTHFR enzymes with non-wild type polymorphic genotypes become thermo-labile, causing a loss of its activity with increased temperature. The modified protein loses its cofactor 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 (4).

In 2003, the association between single nucleotide polymorphisms (SNPs) of *MTHFR* and lung cancer susceptibility was firstly examined in a Taiwan population, indicating that C677T is not associated with lung cancer risk (5). 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 (controls/cases=436/218) in Taiwan, and investigated the interaction of *MTHFR* genotypes and smoking habits in a Taiwanese prostate cancer population.

Materials and Methods

Study population and sample collection. Two hundred and eighteen patients diagnosed with prostate cancer were recruited at the outpatient clinics of general surgery between 2003-2009 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. Twice as many of non-prostate 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 and they were recorded. 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 (6-14). The PCR cycling conditions were: one cycle at 94^{o}C for 5 min; 35 cycles of 94^{o}C for 30 s, 55^{o}C for 30 s, and 72^{o}C for 30 s; and a final extension at 72^{o}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 individuals with complete SNP data and smoking status (cases/controls =218/436) 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* SNP in the controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's Chi-square 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 218 prostate cancer patients and 436 controls are shown in Table II. These characteristics of patients and controls are all well matched. None of the differences between groups were statistically significant (P>0.05) (Table II).

The frequency of the genotypes for the *MTHFR* C677T and A1298C in controls and prostate cancer patients is shown in Table III. The genotype distribution of the genetic polymorphisms of *MTHFR* C677T was significantly different between prostate cancer and control groups (P=0.0051), while that for A1298C polymorphisms was not significant (P>0.05) (Table III). The data indicated that only the *MTHFR* C677T polymorphism was significantly associated with prostate cancer. The frequency of the alleles for *MTHFR* C677T and A1298C in controls and prostate cancer patients is shown in Table IV. The C allele of the *MTHFR* C677T polymorphism was significantly associated with prostate cancer (P=0.0011). The conclusion deduced from Tables III and IV is that the *MTHFR* C677T T allele seems to be associated with a lower risk for prostate cancer in Taiwan.

The interaction between MTHFR genotype and individual smoking habits was further analyzed. The genotype distribution of *MTHFR* C677T was significantly different between prostate cancer patients and controls who have smoking habit (P=0.0004) (Table V), while that for *MTHFR* A1298C was not significant (P >0.05)

(data not shown). The T allele frequency was significantly lower in cancer patients who smoked than in controls. The frequency of individuals with *MTHFR* C677T CT or TT who smoked were approximately 0.5-fold lower than those with CC and prostate cancer than those who did not smoke.

Discussion

In order to investigate the role of *MTHFR* and to find potential biomarkers of prostate cancer, we selected two SNPs of the *MTHFR* gene and investigated their associations with the susceptibility for prostate cancer in a population of central Taiwan. The C677T *MTHFR* polymorphism has been related to acute leukemia (15), endometrial carcinoma (16), and colon adenocarcinoma (17, 18). We found that the T variant genotypes of *MTHFR* C677T were significantly associated with a lower susceptibility for prostate cancer (Tables III and IV). The conclusion was inconsistent with previous findings. Heijmans (19) reported that the incidence of prostate cancer was higher among men with the Val/Val genotype, but others found no such differential distribution between this gene alone and in combinations with other genes (20-22). On the other hand, Van Guelpen and his colleagues (22) after adjusting for serum levels of folates, vitamin B12 and homocysteine, reported that there was a positive association between the heterozygote C677T and the risk of prostate cancer risk. The wide inconsistency may be caused by differences in ethnicity and population. More importantly, a limited sample size may also cause the variation.

We have further analyzed the association between C677T genotype and prostate cancer risk in patients and controls who have cigarette smoking habits. Interestingly, the interaction between *MTHFR* C677T and cigarette smoking habit is clear, *i.e.* smoking people with the CT or TT genotype have a 2-fold reduction in the odds of the

prostate cancer than those smoking people with the CC genotype (Table V).

To sum up, this is the first study which focuses on the SNPs of *MTHFR* and their joint effects with smoking habit on prostate cancer risk in Taiwan, and the presence of the C allele of C677T was associated with a higher risk of prostate cancer. The C allele of *MTHFR* C677T may be a useful marker in prostate oncology for anticancer application, and early cancer detection. In order to further elucidate the importance of the *MTHFR* C677T in prostate carcinogenesis, larger studies assessing circulating levels, as well as dietary intake of folate, are warranted in the future.

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

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

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

Table II. Characteristics of prostate cancer patients and controls.

Characteristic	Controls (n = 436)			Patients (n = 218)			*P*-value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			63.9 (6.6)			63.6 (6.9)	0.58
<50	275	63.1%		142	65.1%		0.67
≥50	161	36.9%		76	34.9%		
Habit							
Cigarette smokers	336	77.0%		177	81.2%		0.27
Non-smokers	100	23.0%		41	18.8%		

[a] Based on Chi-square test.

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

Genotype	Controls		Patients		*P*-value[a]
	n	%	n	%	
C677T rs1801133					**0.0051**
CC	221	50.7%	139	63.8%	
CT	177	40.6%	68	31.2%	
TT	38	8.7%	11	5.0%	
A1298C rs1801131					0.6209
AA	287	65.8%	138	63.3%	
AC	135	31.0%	70	32.1%	
CC	14	3.2%	10	4.6%	

[a] Based on Chi-square test.

Table IV. Distribution of *MTHFR* alleles among the prostate cancer patient and control groups.

Allele	Controls		Patients		*P*-value[a]
	n	%	n	%	
C677T rs1801133					**0.0011**
Allele C	619	71.0%	346	79.4%	
Allele T	253	29.0%	90	20.6%	
A1298C rs1801131					0.4001
Allele A	709	81.3%	346	79.4%	
Allele C	163	18.7%	90	20.6%	

[a] Based on Chi-square test.

Table V. *MTHFR* C677T genotype and prostate cancer after stratification by smoking habit.

Variable	*MTHFR* C677T genotype			
	CC	CT+TT	*P*-value[a]	OR (95% CI)
Smokers			**0.0004***	
Controls	164	172		1.00
Patients	116	61		**0.501 (0.344-0.731)***
Non-smokers			0.9218	
Controls	57	43		1.00
Patients	23	18		1.037 (0.499-2.159)

[a] Based on Chi-square test. OR odds ratio. *Statistically identified as significant.