RESEARCH NOTE

Association of polymorphisms in folate metabolic genes and prostate cancer risk: a case–control study in a Chinese population

DAWEI CAI[1], LIN NING[2], CHUNYU PAN[1], XUEFENG LIU[1], RENGE BU[1], XIAONAN CHEN[1], KEFENG WANG[1], YANG CHENG[3] and BIN WU[1]*

[1]*Department of Urology, Shengjing Hospital of China Medical University, Sanhao Street 36, Shenyang 110004, People's Republic of China*
[2]*Department of Ophthalmology, The General Hospital of Shenyang Military Region, Wenhua Road 83, Shenyang 110016, People's Republic of China*
[3]*Department of Surgery, Shenyang No.1 People's Hospital, Qingquan Road 69, Shenyang 110041, People's Republic of China*

Introduction

Epidemiological studies have shown an association between low folate intake and an increased cancer risk. Folate deficiency is thought to increase the risk of cancer through impaired DNA repair synthesis and disruption of DNA methylation (Duthie 1999; Choi and Mason 2000; Wei *et al*. 2003; Shen *et al*. 2005). However, the reports of association between folate status and risk of prostate cancer are conflicting. Figueiredo *et al*. (2009) reported that daily supplementation with 1 mg of folic acid was associated with an increased risk of prostate cancer in a double-blind randomized clinical trial, whereas Pelucchi *et al*. (2005) found a significant inverse association between dietary folate and prostate cancer risk in a case–control study. The complex role of folate in prostate carcinogenesis may be due to polymorphisms in genes encoding folate metabolism enzymes. We hypothesized that polymorphisms in key folate metabolism genes may influence the effect of folate on prostate cancer development.

Methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR) play important and interrelated roles in the folate metabolic pathway. MTHFR catalyses the reduction of 5,10-methylenetetrahydrolate to 5-methyltetrahydrofolate, the major circulatory form of folate in the body and a carbon donor for the conversion of homocysteine to methionine (Bailey and Gregory 1999). As a precursor of S-adenosylmethionine (SAM), methionine is the universal methyl donor for DNA methylation. MTHFR is also involved in dTMP production and plays a role in DNA synthesis. It has been demonstrated that the C677T and A1298C are two common polymorphisms in the *MTHFR* gene affecting enzyme activity (Frosst *et al*. 1995; Weisberg *et al*. 1998). *MTHFR* 677TT genotype carriers have approximately 30% of the enzyme activity *in vitro* as compared with the *MTHFR* 677CC genotype, and *MTHFR* 677CT genotype show nearly 65% of normal enzyme activity (Frosst *et al*. 1995). The *MTHFR* A1298C variant results in a decrease in MTHFR enzymatic activity that is more pronounced in homozygotes (C/C) than heterozygotes (A/C), although it does not result in a thermolabile protein (Weisberg *et al*. 2001).

MTR catalyses the remethylation of homocysteine to methionine, which is essential for maintaining adequate intracellular methionine and normal homocysteine concentrations (Marchal *et al*. 2008). MTRR catalyses the regeneration of methylcobalamin, a cofactor of MTR. Thus, MTR activity is maintained by MTRR. The polymorphisms of *MTR* A2756G and *MTRR* A66G result in homocysteine elevation and DNA hypomethylation (Leclerc *et al*. 1996).

In this study, we conducted a hospital-based case–control study of the polymorphism of the *MTHFR*, *MTR* and *MTRR* genes and prostate cancer risk in a Chinese population. To our knowledge, this is the first study to investigate the association between the polymorphisms of *MTHFR*, *MTR* and *MTRR* genes and risk of prostate cancer in a Chinese population.

*For correspondence. E-mail: cmu.wubin@yahoo.com.cn; cmu.wubin@hotmail.com.
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Materials and methods

Study population

In the hospital-based case–control study we used 217 prostate cancer cases (mean age: 72.36 ± 12.16 years) and 220 benign prostatic hyperplasia (BPH) controls (mean age: 72.83 ± 12.27 years). We chose the BPH patients as the controls since we could not find many healthy controls without BPH. Prostate cancer patients and BPH patients are all associated with old age and androgen. In addition, most of the men at such high age suffer from BPH. All subjects were genetically unrelated ethnic Han Chinese and were from Shenyang city and its surrounding regions in northeast China. According to histopathology, cases that were newly diagnosed with incident prostate cancer were consecutively recruited between November 2006 and March 2009 from the Shengjing Hospital of China Medical University, without restrictions of age and tumour stage. Those patients who had previously undergone radiotherapy or chemotherapy were excluded. The BPH controls had been given a rectal examination (compatible with BPH) and blood PSA analysis (≤ 4 ng/mL). Control subjects were frequency matched to cases on the basis of age (±5 years). After interview, approximately 5 mL peripheral blood samples were collected in tubes along with EDTA (pH 8) from each participant. This study was approved by the institutional review boards of China Medical University.

Genotyping

MTHFR, MTR and MTRR play important and interrelated roles in folate metabolic pathway. Associations between the polymorphisms of the *MTHFR*, *MTR* and *MTRR* genes and the risk of prostate cancer have been investigated in several studies (Leclerc *et al*. 1996; Heijmans *et al*. 2003; Cicek *et al*. 2004; Singal *et al*. 2004; Van Guelpen *et al*. 2006; Johansson *et al*. 2007; Reljic *et al*. 2007; Marchal *et al*. 2008), but the results have been inconsistent.

The *MTHFR* C677T (rs1801133) and A1298C (rs1801131), *MTR* A2756G (rs1805087) and *MTRR* A66G (rs1801394) polymorphisms were determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers used for *MTHFR* C677T were forward: 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and reverse: 5′-AGGACGGTGCGGTGAGAGTG-3′. The primers used for *MTHFR* A1298C were forward: 5′-AAGGAGGAGCTGCTGAAGATG-3′ and reverse: 5′-CTTTGCCATGTCCACAGCATG-3′. The primers used for *MTR* A2756G were forward: 5′-TGTTCCCAGCTGTTAGATGAAAATC-3′ and reverse: 5′-GATCCAAAGCCTTTTACACTCCTC-3′. The primers used for *MTRR* A66G were forward: 5′-CAGGCAAAGGCCATCGCAGAAGACAT-3′ and reverse: 5′-CACTTCCCAACCAAAATTCTTCAAAG-3′. When digested with *Hinf*I, *MTHFR* 677CC produced one band of 198 bp and *MTHFR* 677TT produced two bands of 175 and 23 bp. When digested with *Mbo*II, *MTHFR* 1298AA produced three bands of 182, 28 and 27 bp, MTHFR 1298CC produced two bands of 210 and 27 bp. When digested with *Hae*III, *MTR* 2756AA produced one 211-bp band, *MTR* 2756GG produced two bands of 131 and 80 bp. When digested with *Nde*I, MTRR 66AA produced two bands of 124 and 27 bp, *MTRR* 66GG produced one 151-bp band. The digested fragments were separated by 3% agarose gel electrophoresis and visualized under UV light.

Statistical analysis

Statistical significance of the differences in the frequency of genotypes using the chi-square test, or Fisher's exact test, odds ratio (OR) and 95% confidence intervals (CI) were calculated when it was appropriate to assess the relative risk conferred by a particular allele and genotype. Hardy–Weinberg equilibrium was tested for goodness-of-fit chi-square test with one degree of freedom to compare the observed genotype frequencies among the subjects with the expected genotype frequencies. To test linkage disequilibrium between *MTHFR* C677T and A1298C polymorphisms, the parameter D' was calculated using the Haploview software (MIT/Harvard Broad Institute, Cambridge, USA). All the other statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) version 11.0 statistical software (SPSS, Chicago, USA). A P value of < 0.05 was considered to be statistically significant.

Results

Cases and controls were well matched in terms of age (±5 years) and ethnicity. Mean age was 72.36 ± 12.16 years for cases and 72.83 ± 12.27 years for controls. There were no significant differences in the frequency distributions of age between cases and controls ($P = 0.981$).

Genotype and allele frequencies distributions for the *MTHFR* C677T and A1298C, *MTR* A2756G and *MTRR* A66G among cases and controls and their associations with risk of prostate cancer are shown in table 1. The genotype frequencies for all the polymorphisms were in agreement with the Hardy–Weinberg equilibrium in controls: *MTHFR* C677T ($P = 0.076$), *MTHFR* A1298C ($P = 0.540$), *MTR* A2756G ($P = 0.139$) and *MTRR* A66G ($P = 0.479$). The frequency of T allele of *MTHFR* C677T was 0.47 for the cases and 0.53 for the controls. The frequency of C allele of *MTHFR* A1298C was 0.16 for cases and 0.18 for controls. The frequency of G allele of *MTR* A2756G was 0.09 for cases and 0.08 for controls. The frequency of G allele of *MTRR* A66G was 0.28 for cases and 0.26 for controls. For the *MTHFR* C677T polymorphism, the difference in the frequencies of genotypes and alleles between cases and controls were statistically significant ($P = 0.043$ for genotypes and $P = 0.021$ for alleles). However, no significant difference for the *MTHFR* A1298C, *MTR* A2756G and *MTRR* A66G polymorphisms were found between cases and controls.

Table 1. Genotype and allele frequencies of the *MTHFR, MTR* and *MTRR* polymorphisms among the cases–controls and the associations with risk of prostate cancer.

Polymorphisms	Cases (n = 217) n (%)	Controls (n = 220) n (%)	P value	OR (95%CI)
MTHFR C677T				
CC	58 (26.73)	45 (20.45)	0.043	1.00[a]
CT	121 (55.76)	116 (52.73)		0.81 (0.51–1.29)
TT	38 (17.51)	59 (26.82)		0.50 (0.28–0.88)
Alleles				
C	237 (53.37)	206 (46.82)	0.021	1.00[a]
T	197 (46.64)	234 (53.18)		0.73 (0.56–0.96)
MTHFR A1298C				
AA	150 (69.12)	144 (65.45)	0.708	1.00[a]
AC	63 (29.03)	71 (32.27)		0.85 (0.57–1.28)
CC	4 (1.84)	5 (2.27)		0.77 (0.20–2.92)
Alleles				
A	363 (83.64)	359 (81.59)	0.424	1.00[a]
C	71 (16.36)	81 (18.41)		0.88 (0.61–1.23)
MTR A2756G				
AA	185 (85.25)	188 (85.45)	0.748	1.00[a]
AG	27 (12.44)	29 (13.18)		0.95 (0.54–1.66)
GG	5 (2.30)	3 (1.36)		1.69 (0.40–7.19)
Alleles				
A	397 (91.47)	405 (92.05)	0.759	1.00[a]
G	37 (8.53)	35 (7.95)		1.08 (0.67–1.75)
MTRR A66G				
AA	111 (51.15)	118 (53.64)	0.869	1.00[a]
AG	92 (42.40)	89 (40.45)		1.10 (0.74–1.62)
GG	14 (6.45)	13 (5.91)		1.15 (0.52–2.54)
Alleles				
A	314 (72.35)	325 (73.86)	0.614	1.00[a]
G	120 (27.65)	115 (26.14)		1.08 (0.80–1.46)

[a]Reference category; OR, odds ratio; CI, confidence interval.

As shown in table 1, the *MTHFR* 677TT genotype had decreased risk of prostate cancer (OR = 0.50, 95%CI: 0.28–0.88) compared with 677CC genotype; the 677T allele was more likely to exert a protective effect on prostate cancer risk (OR = 0.73, 95%CI: 0.56–0.96). However, the *MTHFR* A1298C, *MTR* A2756G and *MTRR* A66G polymorphisms were not significantly associated with the risk of prostate cancer.

The two *MTHFR* polymorphisms were in strong linkage disequilibrium (D' = 0.976), suggesting that there might be haplotype effects among these two variants. As shown in table 2, there were four possible *MTHFR* haplotypes derived from the observed genotypes. Compared with the common haplotype 677C-1298A, the 677T-1298A haplotype was inversely associated with prostate cancer (OR = 0.65, 95% CI = 0.48–0.88).

Discussion

In this study, we have undertaken a case–control study to investigate the role of *MTHFR* (C677T and A1298C), *MTR* A2756G and *MTRR* A66G polymorphisms in susceptibility to prostate cancer in P. R. China. Both cases and controls belonged to the same ethnic background and all shared a common geographic origin in north China.

In the recent years, interest in the genetic susceptibility to cancers has led to a growing attention to the study of polymorphisms of genes involved in tumourigenesis. MTHFR, MTR and MTRR are enzymes that play a central role in the methyl group metabolic pathway, that are involved in both DNA methylation and DNA synthesis. The association between the polymorphisms of the *MTHFR*, *MTR* and *MTRR* genes and prostate cancer risk has been examined in many studies, but the results are inconsistent.

For example, Johansson *et al*. (2007) and Reljic *et al*. (2007) found that *MTHFR* C677T genotypes do not contribute to susceptibility to prostate cancer. Cicek *et al*. (2004) also found no association between the *MTHFR* C677T variant and prostate cancer but found a slight positive association between the *MTHFR* A1298C variant and risk of prostate cancer. Heijmans *et al*. (2003) reported that the incidence of prostate cancer was higher among individuals with the *MTHFR* 677TT genotype. After adjusting for serum levels

Table 2. Frequencies of the *MTHFR* haplotypes among the cases–controls and the associations between the *MTHFR* haplotypes and risk of prostate cancer.

	Cases (n = 434 alleles)	Controls (n = 440 alleles)		
Haplotypes	n (%)	n (%)	P value	OR (95% CI)
677C-1298A	166 (38.25)	127 (28.86)	0.011	1.00[a]
677C-1298C	71 (16.36)	79 (17.95)		0.69 (0.46–1.02)
677T-1298A	197 (45.39)	232 (52.73)		0.65 (0.48–0.88)
677T-1298C	0	2 (0.4)		ND

[a]Reference category; OR, odds ratio; CI, confidence interval; ND, not defined.

of folates, vitamin B12 and homocysteine, Van Guelpen *et al*. (2006) showed that there was a positive relation between the *MTHFR* 677CT and the risk of prostate cancer. Marchal *et al*. (2008) found that there is a strong relation between *MTHFR* 677CT genotype and the risk of prostate cancer, and there is a decreased risk of prostate cancer in *MTHFR* 677TT genotype, but there is no association between the *MTHFR* A1298C, *MTR* A2756G and *MTRR* A66G polymorphisms and risk of prostate cancer.

Recently, Collin *et al*. (2009) conducted a meta-analysis on 13 studies, in which the folate-pathway SNPs did not have significant effects on susceptibility to prostate cancer. The discrepancy of results may be due to the difference in ethnic background and environmental exposure as well as dietary intake of folate, smoking status and use of alcohol. In addition, in our study, we chose only the BPH patients as the controls, because prostate cancer patients and BPH patients are all associated with old age and androgen.

In this study, we found individuals carrying the *MTHFR* 677TT genotype were associated with a reduced risk of prostate cancer in a Chinese population. We also found that the 677T allele was more likely to exert a protective effect on prostate cancer risk. In addition, the 677T-1298A haplotype was inversely associated with prostate cancer. The *MTHFR* 677TT genotype would lead to high 5,10-methylenetetrahydrofolate concentrations, which may provide one more carbon groups for thymidylate synthesis, thereby enhancing DNA synthesis and repair ability (Suzuki *et al*. 2007). The *MTHFR* 677TT genotype would also reduce the risk of an inadequate synthesis of SAM that could provoke a decrease in DNA methylation, favouring the expression of suppressor genes (Marchal *et al*. 2008). Hypermethylation of the CpG promoter sequences of the tumour suppressor genes is probably the most frequent alteration in prostatic carcinoma cells and may be associated with prostatic tumorigenesis (Nelson *et al*. 2003). Moreover, several studies have suggested that whether the *MTHFR* 677T allele is a protective factor or a risk factor depends on dietary folate intake. Unfortunately, the present study did not include data on dietary intake, and thus it was not possible to address the role of folate intake in the current results.

This study has several potential limitations. We have collected the demographical and clinical information such as family history, smoking status, alcohol use and dietary intake of folate of prostate cancer cases, but we have not collected the demographical and clinical information of all controls into statistical analysis, since most of the controls were outpatient.

In conclusion, our findings suggest that the *MTHFR* 677TT genotype and the 677T-1298A haplotype might provide protective effects against prostate cancer risk.

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