Folate metabolism-related gene polymorphisms and susceptibility to primary liver cancer in North China

Lian-Hua Cui · Yang Song · Hongzong Si · Fangzhen Shen · Min-Ho Shin · Hee Nam Kim · Jin-Su Choi

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Abstract Genetic factors may contribute to individual differences in cancer susceptibility. This study was designed to investigate the effects of the polymorphisms of methylenetetrahydrofolate reductase 677 C → T (MTHFR 677 C → T), methylenetetrahydrofolate reductase 1298 A → C (MTHFR 1298 A → C), thymidylate synthase (TYMS 3R → 2R), and methionine synthase 2756 A → G (MTR 2756 A → G) on the risk of primary liver cancer (PLC). We conducted a case–control study involving 356 PLC cases and 641 healthy controls in North China. Compared with the MTHFR 677CC genotype, the MTHFR 677TT genotype showed an increased risk for PLC (TT vs. CC: adjusted odds ratio (OR) = 1.56; 95% confidence interval (CI): 1.02–2.40; \( P = 0.043 \)) after adjusting for gender and age, whereas the MTHFR 1298CC genotype showed a significantly decreased risk for PLC (CC vs. AA: adjusted OR = 0.23; 95% CI: 0.08–0.70; \( P = 0.010 \)). However, no significant association was found between the TYMS 3R → 2R or the MTR 2756 A → G polymorphism and the risk of PLC. Our results suggest that the MTHFR 677 C → T and the MTHFR 1298A → C genetic polymorphisms might play important role in hepatic carcinogenesis. Further studies with larger sample sizes are required to validate this association.

Keywords Methylenetetrahydrofolate reductase 677 C → T polymorphisms · Methylenetetrahydrofolate reductase 1298 A → C · Thymidylate synthase polymorphism · Methionine synthase 2756 A → G polymorphisms · Primary liver cancer · Susceptibility

Background

Primary liver cancer (PLC) is a common cause for cancer-related death worldwide. About 82% of cases (and deaths)
occur in developing countries, and China accounts for 55% of all PLC deaths in the world [1]. Established or probable risk factors for PLC include chronic infections with the hepatitis B virus (HBV) and/or hepatitis C virus (HCV), dietary exposure to aflatoxin B1, and heavy alcohol consumption; however, genetic susceptibility factors for PLC have not been examined extensively. The liver is the main site for storage and metabolic handling of all vitamins, including vitamins folates, B12, and B6 and which function as key cofactors for metabolic activity of enzymes [2].

Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in folate metabolism. A common mutation of the MTHFR gene is the C to T transition at nucleotide 677, which converts alanine to valine, results in a thermolabile enzyme with decreased activity [3]. Individuals with the MTHFR 677TT or CT genotype have an approximately 30 or 65% of the enzyme activity in comparison with those possessing the 677CC wild-type [4]. A lower MTHFR activity has been shown to be associated with DNA hypomethylation, genomic instability, and derepression of proto-oncogenes [3]. A second less common polymorphism in the MTHFR gene results in a glutamate to alanine (A → C) change at position 1298 leading to mildly decreased MTHFR activity [5]; nevertheless, this variant has not associated either with a thermolabile enzyme or with alterations in the levels of homocysteine in the plasma [5].

Thymidylate synthase (TYMS) catalyses the conversion of deoxyuridine monophosphate (dUMP)–deoxythymidine monophosphate (dTMP) using 5,10-methylenetetrahydrofolate as the methyl donor in DNA synthesis. The TYMS gene has a two or three 28 bp tandem repeat sequence in the 5′-untranslated region, the number of this repeats being polymorphic. In vitro, compared with the 2R, the 3R has been associated with greater TYMS expression and enzyme activity [6]; impairments of this enzyme may result in chromosomal breakage and fragile site induction, which may cause individual susceptibility to cancer.

Methionine synthase (MTR), a vitamin B12-dependent enzyme, also plays an important role in folate metabolism through the enzymatic conversion of homocysteine to methionine, which is required for the production of S-adenosylmethionine (SAM), the major methyl group donor in DNA methylation. The gene polymorphism in MTR 2756 A → G leads to a change from aspartic acid to glycine and has been predicted to alter enzyme activity [7], which may affect DNA methylation processes [8].

Regarding the folate pathway enzyme gene polymorphisms in relation to PLC risk, prior studies have focused mainly on the influence of the MTHFR 677 C → T polymorphism on risk of PLC; however, results remain conflicting rather than conclusive [9–15]. For the MTHFR 1298 A → C polymorphism, three studies have reported an association between the MTHFR 1298 A → C polymorphism and risk of hepatocellular carcinoma with conflicting results [14, 15]. Additionally, no prior report exists regarding MTR 2756 A → G and TYMS 3R → 2R polymorphism and PLC risk. Thus, we conducted the present study to examine the potential role of MTHFR 677 C → T, MTHFR 1298 A → C, TYMS 3R → 2R, and MTR 2756 A → G polymorphisms on PLC risk in North China.

Methods

Subjects

The study population consisted of 356 patients with newly diagnosed PLC and 641 population-based controls. All enrolled patients were pathologically or clinically confirmed at Affiliated Hospital of Medical College Qingdao University from 2008 to 2009. Patients must be newly diagnosed, and cases with secondary or recurrent tumour were excluded.

The control group was recruited from a Nutritional Survey, which was designed to investigate the nutritional status of Chinese adults who lived in a residential community of Qingdao City of Shandong Province, China. The Nutritional Survey base for health examination was conducted from 2008 to 2009. Among the 7 residential communities (approximately 7,500 households) in Qingdao, 1,080 households (approximately 7 household intervals) were selected by using systematic sampling method and only 1 participant was selected from each household. One thousand and two eligible subjects aged 25 years and older were invited by letter or telephone to participate in this study. We excluded the individuals with a history of cancer or major organ failure (e.g., heart, brain, lung, kidney, or liver), and a total of 641 healthy individuals (response rate 64%, 279 men, 362 women) with a median age of 58.84 years were recruited as controls. All subjects underwent clinical examinations and answered a questionnaire regarding lifestyle, medical and medication history, and, for women, menopausal status. At the time of their peripheral blood collections, all case and control subjects provided their informed consent. This study was approved by the Institutional Review Board of the Affiliated Hospital of Medical College Qingdao University, China.

Genotyping

Genomic DNA was extracted from peripheral blood using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s protocol. Genotyping for the detection of the MTHFR C677T and MTHFR
A1298C polymorphism was performed by real-time polymerase chain reaction (PCR), using dual-labelled probes containing locked nucleic acids (LNA), in a real-time PCR assay [16]. PCR primers and LNA probes were designed and synthesized by Integrated DNA Technologies (IDT, Coralville, IA, USA).

Genotyping for 28 bp tandem repeat (3R → 2R) genotypes in the 5’-UTR of TYMS gene was conducted using a protocol described by Horie et al.[17]. The TYMS SNP was genotyped by digesting the TYMS VNTR PCR products with HaeIII (Takara, Otsu, Japan) followed by 6% polyacrylamide gel electrophoresis (PAGE).

Genotyping for the detection of the MTR 2756 A → G polymorphism was performed using specific oligonucleotide primers [8, 16]. Digestion products with HaeIII (Takara) were visualized after electrophoresis in a 10% polyacrylamide gel (19:1) using the MADGE system.

Statistical analyses

The demographic characteristics of the PLC patients and the controls were compared using the chi-square test. The reported genotype frequencies in the control group were checked for accordance with Hardy–Weinberg equilibrium. Unconditional logistic regression analysis was conducted to estimate odds ratios (ORs) and their 95% confidence intervals (95% CIs) for associations between each gene polymorphism (MTHFR 677 C → T, MTHFR 1298 A → C, TYMS 3R → 2R, and MTR 2756 A → G) and PLC risk. All tests of association were adjusted for age and gender. All analyses were performed using the Statistical Package for the Social Sciences software version 15.0 (SPSS, Chicago, IL, USA).

Results

Table 1 shows characteristics of the study population. In total, 356 cases and 641 controls were included in these analyses.

The cancer group comprised 296 male and 60 female patients, while the control group consisted of 279 men and 362 women. A statistically significant gender difference was also found between patients with PLC and healthy controls; the control group had more female subjects ($P < 0.01$). The mean age was 56.6 ± 10.4 years for the cases and 58.7 ± 9.8 years for the controls ($P < 0.05$), and there was no significant differences on the distribution of 5-year strata of age groups between cancer groups and control groups (data not shown, $P > 0.05$). The proportion of drinking in PLC cases was higher than in the controls ($P < 0.01$).

Table 2 shows genotype distributions for MTHFR 677 C → T, MTHFR 1298 A → C, TYMS 3R → 2R, and MTR 2756 A → G and their adjusted ORs and 95% CIs for PLC risk. The genotype frequencies for all the polymorphisms in the controls were in agreement with the Hardy–Weinberg equilibrium.

Compared with the MTHFR 677CC genotype, the MTHFR 677CT genotype showed an increased risk for PLC (TT vs. CC: adjusted OR = 1.56; 95% CI: 1.02–2.40; $P = 0.043$) after adjusting for gender and age, whereas the

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<th>Table 1 Demographic characteristics of subjects</th>
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<td><strong>PLC n (%)</strong></td>
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*SD standard deviation
*PLC compared with control
MTHFR 1298CC genotype showed a significantly decreased risk for PLC (CC vs. AA: adjusted OR = 0.23; 95% CI: 0.08–0.70; \( P = 0.010 \)). However, no significant association was found between the TYMS 3R ? 2R or MTR 2756 A ? G polymorphism and the risk of PLC.

**Discussion**

The current study evaluated a potential association between the folate metabolism-related gene polymorphisms (MTHFR 677 C ? T, MTHFR 1298 A ? C, TYMS 3R ? 2R, and MTR 2756 A ? G) and susceptibility to PLC in North China. We found weak evidence that the MTHFR 677TT genotype was associated with increased risk of PLC after adjusting for gender and age. By contrast, the MTHFR 1298CC genotype showed a significantly decreased risk for HCC, and we failed to identify any significant major effects of the TYMS 3R ? 2R or MTR 2756 A ? G polymorphisms on overall PLC risk.

Regarding the MTHFR 677 C ? T polymorphism, results of several studies examining the role of this polymorphism in PLC susceptibility have been inconsistent. Four studies have shown that the MTHFR 677CT or TT genotype was associated with increased risk of hepatocellular carcinoma [9–12]. By contrast, Yuan et al. [14] in the United States showed that individuals with the MTHFR 677TT genotype had decreased risk of hepatocellular carcinoma. Saffroy et al. [13] in France showed that the MTHFR 677CT or TT genotype decreased the risk of developing hepatocellular carcinoma. However, another study in Korea observed no association between the MTHFR 677 C ? T polymorphism and genetic susceptibility to hepatocellular carcinoma [15]. In our study, individuals with the MTHFR 677TT genotype showed a weak tendency for an increased risk of PLC. Our results are similar to a recent meta-analysis by Jin et al. [18], which was based on eight case–control studies and suggested that the MTHFR 677 C ? T polymorphism increased the risk of hepatocellular carcinoma in an overdominant model and might be a risk factor for hepatocellular carcinoma occurrence.

The cancer risk associated with MTHFR polymorphisms has been suggested to be modulated by folate intake. Under adequate folate conditions, the protective effect of the 677TT genotype is associated with elevated risk of cancer among MTHFR 677TT genotypes with low folate intakes [19]. Our study was conducted in a northern Chinese population with a lower serum folate level due to low consumption of vegetables and fruit in North China, as reported by Hao et al. [20]. A combination of the MTHFR 677TT genotype and inadequate folate levels might result in global DNA hypomethylation that is a feature of early tumorigenesis and is associated with the development of cancer [21, 22].

In our study, the MTHFR 1298CC genotype was more frequent among controls than among cases. Additionally, we also observed a significant association of the MTHFR1298 A ? C polymorphism with PLC in a multivariate analysis after adjusting for confounding risk.
factors, including age and gender. Our study suggested that the 1298C variant allele might exert a protective effect against PLC. To date, only three studies have investigated the role of the MTHFR 1298 A → C polymorphism in the risk of PLC; however, the results have been inconsistent [9, 14, 15]. Yuan et al. [14] reported that individuals with homozygous mutant alleles at either the 677 or 1298 locus of the MTHFR gene showed reduced risk of PLC compared with those with homozygous wild-type alleles. By contrast, in a Korean case–control study of hepatocellular carcinoma, the MTHFR 1298A → C polymorphism were associated with an increased risk of hepatocellular carcinoma [15]. However, another study in South China observed no association with risk of primary hepatocellular carcinoma [9].

To our knowledge, no prior report exists regarding the MTR 2756 A → G and TYMS 3R → 2R polymorphisms and PLC risk. MTR is involved in the pathway of remethylation of homocysteine to methionine, thus playing a critical role in maintaining adequate intercellular S-adenosyl-methionine (SAM) levels for DNA methylation, which has a cancer-suppressor effect. The MTR gene harbors an A2756G polymorphism, which is thought to affect the enzymatic activity that may affect DNA methylation processes. However, the role of the MTR2756 A → G polymorphism in cancer risk appears controversial. A recent meta-analysis by Yu et al. [23] suggested that 2756GG was associated with a significantly reduced risk of many types of cancer (excluding liver cancer) in European populations. However, in Asian populations, a significantly elevated association between the 2756GG genotype and cancer risk (excluding liver cancer) was observed. Our finding of no association between the MTR 2756 A → G polymorphism and risk of PLC is similar to previous reports in other cancer types, such as non-Hodgkin lymphoma [16] and lung cancer [24, 25].

TYMS is also involved in folate metabolism and catalyzes methylation of dUMP to dTMP, which is essential for DNA replication. The TYMS polymorphism is thought to affect TYMS expression activity and protein translation efficiency [6, 26]. Although the effects of TYMS gene polymorphisms on susceptibility to human cancer have been investigated in many studies [16, 27, 28], the TYMS gene polymorphisms have not been evaluated relative to the risk of hepatocellular carcinoma. In the present study, we found no major effect of the TYMS 5' UTR polymorphism on risk of PLC, which is consistent with our previous studies in non-small-cell lung cancer in Northern Chinese [29] and in other tumor types, such as lung cancer [30], breast cancer [31], and multiple myeloma [32].

The limitations of our study must also be acknowledged. Although the overall PLC sample size was not small, the numbers of subjects in each subgroup, such as the female group, was small; thus, we cannot confirm the subgroup analysis.

Conclusions

Our results suggest that the MTHFR 677 C → T and MTHFR 1298A → C genetic polymorphisms may contribute to PLC development in Northern Chinese. The MTHFR 677 TT genotype was found to increase the risk for PLC, whereas the MTHFR 1298 CC genotype was associated with a significantly decreased risk for PLC. Further studies with larger sample sizes are required to validate these associations.

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Conflict of interest The authors declare that they have no conflict of interest.

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