Published in final edited form as:
Hepatology. 2007 September ; 46(3): 749–758.

Genetic Polymorphisms in the *Methylenetetrahydrofolate Reductase* and *Thymidylate Synthase* Genes and Risk of Hepatocellular Carcinoma

Jian-Min Yuan[1], **Shelly C. Lu**[2], **David Van Den Berg**[2], **Sugantha Govindarajan**[2], **Zhen-Quan Zhang**[3], **Jose M. Mato**[4], and **Mimi C. Yu**[1]

1*The Cancer Center, University of Minnesota, Minneapolis, Minnesota*

2*Keck School of Medicine, University of Southern California, Los Angeles, California*

3*Department of Epidemiology, Cancer Institute of Guangxi, Nanning, Guangxi, China*

4*CIC bioGUNE, Ciberehd, Technology Park of Bizkaia, Derio, Bizkaia, Spain*

Abstract

Methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TYMS) are known to play a role in DNA methylation, synthesis, and repair. The genetic mutations in *MTHFR* and *TYMS* genes may have influences on their respective enzyme activities. Data on the association studies of the *MTHFR* and *TYMS* genetic polymorphisms and risk of hepatocellular carcinoma (HCC) are sparse. *MTHFR* and *TYMS* genotypes were determined on 365 HCC cases and 457 healthy control subjects among Hispanic and non-Hispanic whites and African-Americans in Los Angeles County, California, and among Chinese in the city of Nanning, Guangxi, China. Relative to the high-activity genotype, each low-activity genotype of *MTHFR* was associated with a statistically nonsignificant 30% to 50% reduction in risk of HCC. Relative to the *TYMS3′UTR +6/+6* genotype, individuals with 1 or 2 copies of the deletion allele had a statistically significant 50% reduction in risk of HCC. When we examined HCC risk by the total number of mutant alleles in the 3 polymorphic loci of *MTHFR/TYMS* (range, 0-4), there was a monotonic decrease in risk with increasing number of mutant alleles (*P* for trend = 0.003). Individuals possessing the maximum number of mutant alleles (*i.e.*, 4) had an odds ratio of 0.46 (95% confidence interval = 0.23-0.93) for HCC compared with those with no or only 1 mutant allele.

Conclusion—This study supports the hypothesis that reduced MTHFR activity and enhanced TYMS activity, both of which are essential elements in minimizing uracil misincorporation into DNA, may protect against the development of HCC.

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world and the third leading cause of cancer deaths.[1] The overall 5-year survival for patients with HCC is less than 10%. Only 30% of patients with HCC are considered candidates for surgical resection; however, the recurrence rate after surgery is approximately 40% to 50%.[2] The major risk factors for HCC vary somewhat with its geographical distribution. Chronic infection with hepatitis B virus (HBV) is by far the most important risk factor for HCC in humans and is the primary cause of this cancer in high-risk areas, including China and Africa.[3,4] Chronic infection with hepatitis C virus (HCV) is another risk factor for HCC and has an increasingly prominent role in the development of HCC in countries such as the United States where rates

Address reprint requests to: Jian-Min Yuan, Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, 1300 South 2nd Street, Suite 300, Minneapolis, MN 55454. E-mail: jyuan@umn.edu; fax: 612-624-0315.

Potential conflict of interest: Nothing to report.

of infection with HBV are relatively low.[5] Other environmental risk factors for HCC include dietary aflatoxin, which plays a prominent role in high-risk areas such as China and Africa; excessive alcohol intake; cigarette smoking; diabetes; and obesity.[6-8] Given that only a minority of people exposed to these risk factors eventually develop HCC, other cofactors (environmental and/or genetic) are likely to be involved in the development of HCC in humans.

Experimental studies have shown that diets deficient in methyl groups (folate, methionine, and choline) can induce HCC in rodents[9,10] along with an increased level of uracil in DNA and accumulated DNA strand breaks in the *P*53 gene of the hepatocytes.[11-13] Thymidylate is a rate-limiting nucleotide required for DNA synthesis and repair. Thus, a sufficient pool of thymidylate is essential in minimizing the misincorporation of uracil into DNA, chromosomal breakage, and fragile site induction,[14,15] leading to reduced susceptibility to HCC. Thymidylate synthase (TYMS) catalyzes the synthesis of thymidylate or deoxythimidine monophosphate (dTMP) from deoxyuridine monophosphate (dUMP), with 5,10-methylenetetrahydrofolate as the methyl donor (Fig. 1). In humans, a polymorphic 28–base pair (bp) tandem repeat element in the 5′-untranslated promoter enhancer region of the *TYMS* gene (*TYMSER*) has been described.[16] Triple repeat (*3R*) and double repeat (*2R*) alleles are the most common polymorphic alleles.[17] *In vitro*, compared with the *2R*, the *3R* has been associated with greater TYMS expression and enzyme activity.[18] More recently, a second *TYMS* polymorphism, a 6-bp insertion/deletion at nucleotide 1494 in the 3′-untranslated region of the *TYMS* gene (*TYMS3′UTR*) was identified.[19] The functional consequences of this polymorphism are not known, although it was thought to affect TYMS mRNA expression and stability.[19,20] The 2 polymorphisms appear to be in linkage disequilibrium,[19] with the *6-bp deletion* allele linked with the *TYMSER*3R*.[20,21]

Methylenetetrahydrofolate reductase (MTHFR), a key enzyme in 1-carbon metabolism, catalyzes the irreversible conversion of 5,10- methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate. The 5-methyltetrahydrofolate serves as the methyl donor for the remethylation of homocysteine to methionine, the precursor of S-adenosylmethionine (SAMe). SAMe is the principal biological methyl donor for methylation of DNA and other large molecules (Fig. 1). SAMe deficiency can predispose to HCC.[22] Two common genetic polymorphisms in the *MTHFR* gene have been identified.[23,24] One polymorphism, a C-to-T transition at nucleotide 677 (*C677T*), results in an alanine-to-valine conversion in the protein.[23] *In vitro* studies have shown the enzymatic activity levels of *CT* and *TT* genotypes to be 65% and 30%, respectively, of the CC genotype.[25] Another polymorphism, an A-to-C transition located at nucleotide 1298 *(A1298C)* of the gene, results in a glutamate-to-alanine change in the protein residue that is associated with a diminished level of enzyme activity.[24,26] A lower *MTHFR* activity will result in an increased pool of 5,10- methylenetetrahydrofolate for TYMS, which may favor optimal DNA synthesis and repair by reducing uracil misincorporation and double-strand breaks of DNA.[27]

Although abundant literature exists on the associations between the *MTHFR* and *TYMS* polymorphisms and risk of colorectal cancer,[28] much less is known about these genetic polymorphisms in relation to HCC. Given their competition for the common substrate, 5,10-methylenetetrahydrofolate, and their critical role in 1-carbon metabolism, we studied the genetic polymorphisms of *MTHFR* and *TYMS* genes and risk of HCC among low-risk non-Asians in Los Angeles, California, and high-risk Chinese in southern Guangxi, China.

Patients and Methods

The study included participants of 2 case-control studies of HCC, 1 in low-risk non-Asians in Los Angeles, California, and the other in high-risk Chinese in the southern part of the Guangxi Autonomous Region, China. The designs of the 2 studies have been described previously.[8,

[29] Permission to conduct this study had been obtained from the Institutional Review Boards at the University of Southern California and the Guangxi Cancer Institute. Separate informed consent forms for interview and biospecimen collection were obtained from each study participant.

HCC Patients

In Los Angeles, we studied incident HCC in black, Hispanic, and non-Hispanic white residents of Los Angeles County who were between 18 and 74 years of age at diagnosis from January 1984 through December 2001. Cases were identified through the Los Angeles County Cancer Surveillance Program, a population-based cancer registry that records all incident cancers diagnosed in residents of Los Angeles County. Because of the rapidly fatal nature of HCC (the median time interval between diagnosis and death is approximately 3 months), 84% of eligible patients died before our attempted contact. Among the 478 patients we contacted, 34 (7%) were too ill to be interviewed, and 325 (73%) of the remaining 444 were interviewed. An experienced hepatopathologist reviewed the histology slides of all interviewed HCC patients; 25 cases judged to be non-HCC were excluded.

In Guangxi, China, we identified newly diagnosed HCC from 4 major hospitals in the city of Nanning. Participating hospitals were comparable in their quality of patient care and diagnosis. Only patients diagnosed during September 1995 through September 1998, between the ages of 20 and 64 years, and residing in Nanning City or its neighboring townships were asked to participate in the study. We began the study in October 1995 and closed enrollment in October 1998 when 250 patients had been recruited into the study.

Control Subjects

In Los Angeles, we sought to recruit up to 2 control subjects per case from the neighborhoods where HCC patients resided at the time of diagnosis, who were matched to the index case by sex, age (within 5 years), and race (Hispanic white, non-Hispanic white, black). A total of 474 neighborhood control subjects were recruited into the study; most were the first (74%) or second (12%) eligible neighbors.

In Guangxi, China, we identified 1 consenting control subject per case among all patients admitted to the same hospital within 1 month of the index case's hospital admission, who had no history of cancer or clinical liver cirrhosis. The matching criteria were age (within 3 years), sex, ethnicity (Han, Zhuang, Yao, other), and district (if resident of Nanning City) or township (if resident of neighboring townships) of residence.

Data Collection

All consenting cases and control subjects in Los Angeles, California and Guangxi, China were interviewed in person by trained interviewers using structured questionnaires. Both the Los Angeles and Guangxi questionnaires solicited demographic information, lifetime use of tobacco and alcohol, medical history, and other lifestyle factors. An alcohol drinker was defined as someone who had drunk alcoholic beverages at least once a week for 6 months or longer. One drink was defined as 360 g beer (12.6 g ethanol), 103 g wine (12.3 g ethanol), or 30 g spirit (12.9 g ethanol). A smoker was defined as someone who had ever smoked on a daily basis. Smokers were asked at what age (years) they began smoking on a daily basis, the average number of cigarettes smoked per day, and total number of years of smoking. Former smokers were asked about the number of years since smoking cessation.

Serum and buffy coat samples were collected from all subjects of the Guangxi study (250 cases and 250 controls). For the Los Angeles study, we collected from study subjects serum samples beginning in January 1992 and buffy coat samples beginning in October 1995. The buffy coat

samples were available on 120 (73%) of 164 eligible HCC cases (*i.e.*, those interviewed after October 1995). For the 277 control subjects from whom DNA donation was sought, 230 (83%) consented and donated blood samples. We examined and found no differences in the distributions by age, sex, level of education, cigarette smoking, alcohol consumption, history of diabetes, and serological markers for HBV and HCV infections between subjects with DNA (*i.e.*, those included in the current study) and those without DNA, both for the HCC case and control subjects groups.

Laboratory Tests

Blood samples from cases and controls were processed and stored (−20°C) in an identical manner. The assays used for testing serologic markers of HBV and HCV infections have been described previously.[8,29] Briefly, we tested all study samples for the presence of hepatitis B surface antigen in serum using commercialized kits (AUSRIA, Abbott Laboratories, North Chicago, IL), and negative samples (for the Los Angeles study only) were further tested for the presence of antibodies to the hepatitis B core antigen using standard testing kits (Corab, Abbott Laboratories, North Chicago, IL). All samples were tested for the presence of antibodies to the hepatitis C virus (anti-HCV) in serum using the enzyme linked immunosorbent assay version 2.0 kit manufactured by Ortho Diagnostic Systems, with confirmation of positive samples using RIBA version 2.0 (Chiron, Emeryville, CA). Serum samples were tested blindly, identified only by codes without regard to case/control status.

DNA was purified from buffy coats of peripheral blood using a QIAamp 96 Blood Kit (Qiagen, Valencia, CA). Genotyping assays were developed for the *MTHFR* gene polymorphisms (677C→T and 1298A→C) and the *TYMS* 3′UTR polymorphism (*1494 6-bp ins/del*) using the fluorogenic 5′-nuclease assay (TaqMan Assay).[30] The sizes of the *MTHFR* and *TYMS* genes are 7,105 and 1,536 bp, respectively. The TaqMan assays were performed using a TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. The oligonucleotide primers (Integrated DNA Technology, Coralville, IA) for amplification of the polymorphic regions of *MTHFR* and *TYMS* and the fluorogenic oligonucleotide probes (Applied Biosystems) used to detect each of the alleles are listed in Appendix A. Polymerase chain reaction amplification using approximately 10 ng genomic DNA was performed in a thermal cycler (MWG Biotech, High Point, NC) with an initial step of 95°C for 10 minutes, followed by 50 cycles of 95°C for 25 seconds and 1 minute at the annealing temperature (Appendix A). The fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System and the results analyzed with Sequence Detection Software (Applied Biosystems). Experimental samples were compared with 12 standard controls to identify the 3 genotypes at each locus. Any samples that were outside the parameters defined by the controls were identified as noninformative and were retested.

Five HCC cases and 13 controls were noninformative in 1 or more of the 3 tested polymorphisms in *MTHFR* and *TYMS* genes. These subjects were excluded. Thus, the current analysis involved 365 HCC cases and 457 control subjects.

Statistical Analysis

Chi-square test was used to examine differences in the distributions of selected demographic variables between cases and controls, and in genotypic frequencies by race/ethnicity among controls. Unconditional logistic regression models[31] were used to examine the associations between the *MTHFR* and *TYMS* gene polymorphisms and risk of HCC. The strength of a given gene/exposure-HCC association was measured by its odds ratio and the corresponding 95% confidence interval and 2-sided *P* value. Subjects' age, sex, and race/ethnicity were included as covariates in all models. The study location (Los Angeles vs Guangxi) also was included as a covariate in models involving data from both the Los Angeles and Guangxi studies. When

we examined the main effects of genotypes on HCC risk, the following risk factors for HCC were included as covariates in the logistic regression models: number of cigarettes smoked per day, number of alcoholic drinks per day, and seropositivity to markers of HBV and HCV infections.

We examined linkage disequilibrium between the two *MTHFR* gene polymorphisms in control subjects using the Expectation-Maximization algorithm.[32] The measure of the statistical association for linkage disequilibrium, R^2, a better measure for linkage disequilibrium than the commonly used Lewontin's *D*, was used to assess the correlation of alleles at 2 sites.[33]

Statistical analysis was conducted using the SAS software Version 9.1 (SAS Institute, Cary, NC). All *P* values quoted are 2-sided. Two-sided *P* values that are 0.01 or less were considered statistically significant after taking into account for multiple comparisons using the Bonferroni method.[34]

Results

The mean ages (±standard deviation) of HCC patients and control subjects in Los Angeles, California, were 60.4 (±10.3) years and 59.1 (±10.8) years, respectively. The corresponding figures in Guangxi, China were 49.3 (±9.6) and 49.5 (±10.3). In Los Angeles, cases had higher proportions of Hispanic or African-Americans and were less educated than controls (Table 1).

Cigarette smoking, heavy alcohol consumption, and presence of hepatitis B and/or C serology were risk factors for HCC in both the low-risk (Los Angeles, CA) and the high-risk (Guangxi, China) populations (Table 2). Subjects who smoked 20 or more cigarettes per day had a statistically significant 70% increase in risk of HCC compared with nonsmokers, and individuals consuming 4 or more alcoholic drinks per day experienced approximately 4 to 6 times the risk relative to nondrinkers. Chronic infection with HBV was the main viral risk factor for HCC in Guangxi, whereas HCV infection was the more important viral risk factor for HCC in Los Angeles.

Table 3 shows the genotypic and allelic frequencies of the *MTHFR* and *TYMS3'UTR* polymorphisms by study location and race/ethnicity among controls. There were no statistically significant differences in either genotypic or allelic frequencies between non-Hispanic whites and Hispanics/blacks in Los Angeles, California, or between Han and non-Han Chinese in Guangxi, China. Compared with Chinese, non-Hispanic whites or Hispanics/blacks (32 Hispanics and 10 black subjects) had higher frequencies of the *MTHFR-C677T* but lower frequencies of the *TYMS 3'UTR1494del6* polymorphisms (both *P* values for difference in genotype frequencies among the 4 racial/ethnic groups <0.0001). Genotypic and allelic frequencies of the *MTHFR-A1298C* polymorphism were comparable among the 4 racial/ethnic groups. The distributions of all genetic polymorphisms within a race/ethnic population did not statistically significantly deviate from the Hardy-Weinberg equilibrium after taking into account for multiple comparisons (all $P < 0.01$) (Table 3).

Given the differences in allelic frequencies between the racial/ethnic groups under study, we first examined all genotype-risk associations within each racial/ethnic group. No differences in genotype-risk associations across the 4 groups were observed (all *P* values for genotype × race/ethnicity interaction > 0.65) (data not shown). Again, we did not detect any statistically significant difference in genotype–HCC risk associations between the 2 study populations (all *P* values for genotype × study location interaction ≥0.19). Therefore, we presented the results by study location separately and in combination with adjustment for race/ethnicity and study location.

Individuals possessing homozygous mutant alleles at either the 677 or 1298 loci of the *MTHFR* gene had a 30% to 50% reduction in risk of HCC compared with those with homozygous wild-type alleles (Table 4). The alleles at the 2 loci were in linkage disequilibrium (Table 5). Individuals with the *677TT* genotype all had the *1298AA* genotype, whereas those with the *1298CC* genotype all possessed the *677CC* genotype (R^2 for linkage disequilibrium = 0.16, $P < 0.001$). When the 2 genotypes were examined jointly in all study subjects, those possessing the maximum number of mutant alleles, which is 2 (677CC/1298CC or 677TT/1298AA), had the lowest risks for HCC (Table 5). When the total number of mutant alleles (range, 0-2) in the 2 polymorphic loci (C677T and A1298C) was considered, a statistically non-significant decrease in HCC with increasing number of mutant alleles was observed (Table 4).

Table 4 also shows the association between the *TYMS3′UTR1494del6* and HCC risk. The association was slightly stronger in Los Angeles than in Guangxi subjects, although the difference was not statistically significant ($P = 0.63$). When both study populations were combined, individuals with 1 or 2 copies of the 6-bp deletion allele had a statistically significant, 50% reduction in risk of HCC relative to those with the *+6/+6* genotype.

When we summed the number of mutant alleles in both the *MTHFR* and *TYMS* genes, we noted a monotonic, statistically significant decrease in HCC risk with increasing number of mutant alleles (P for trend = 0.003, Table 4). Compared with those with no or only 1 mutant allele, individuals with 3 or 4 mutant alleles exhibited a more than 50% reduction in risk of HCC.

We examined the associations between HCC risk and *MTHFR/TYMS* genotypes stratified by age (younger than 50 years, 50+ years), sex, race (non-Hispanic whites, Hispanics/African-Americans, Chinese), smoking (never vs ever smokers), alcohol use (nondrinkers vs weekly drinkers), hepatitis B/C serology (negative vs positive). No discernible modifying effects by these risk factors for HCC were noted (data not shown).

Discussion

Few epidemiologic studies have examined the role of methylation genotypes in HCC development. The current study is the first to investigate the joint effects of *MTHFR* and *TYMS* genotypes on HCC risk. Our results show that individuals possessing variant alleles of the *MTHFR* and *TYMS* genes had statistically significant decreased risk of HCC.

MTHFR is a key folate-metabolizing enzyme, which catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Both variant alleles of the *MTHFR* gene loci under study have been found to be associated with reduced enzyme activity. The 1298A→C mutation has been shown to influence specific enzyme activity and homocysteine and folate concentrations, but to a lesser extent than the 677C→T mutation. Functional studies have demonstrated that individuals possessing both mutations (*i.e.*, homozygous mutant at either the *677* or *1298* site or heterozygous mutant at both loci) showed the lowest enzyme activities.[26,35] Epidemiologic studies have noted that the *677T* and/or *1298C* genetic variants are in linkage disequilibrium,[35,36] and both genotypes are associated with a reduced risk of colorectal cancer.[28] A recent study reported that individuals with the *MTHFR677CT* or *TT* genotypes had a reduced risk of HCC.[37] Our findings are consistent with all of these prior observations.

Several studies have reported that the *MTHFRC677T* polymorphism can modify the effect of alcohol on risk of colorectal cancer,[38] breast cancer,[39] and esophageal cancer.[40] In the aforementioned study,[37] patients with alcoholic liver cirrhosis possessing at least one *T* allele of the *MTHFR677* polymorphism were less likely to develop HCC compared with their counterparts with the *CC* genotype, indicating a stronger protective effect of *MTHFR* mutation

on alcohol-induced hepatocarcinogenesis. Alcohol (ethanol) can interfere with folate metabolism either directly or through its metabolite acetaldehyde.[41] Alcohol intake along with the *MTHFR* genetic mutation had significant influence on the inverse association between folate intake and plasma homocysteine.[42,43] These findings support the hypothesis that reduced MTHFR enzyme activity resulting from the genetic mutation may protect against the development of HCC, through the increase in the supply of 5,10-methyltetrahydrofolate, a substrate of the TYMS enzyme.

Thymidylate synthase catalyzes the conversion of dUMP to dTMP at the expense of 5,10-methylenetetrahydrofolate, thus reducing uracil misincorporation into DNA and the occurrence of DNA double-strand breaks. Any genetic mutations that lead to a reduced function of the TYMS enzyme may adversely affect DNA synthesis and repair. The current study demonstrates a statistically significant association between *TYMS3′UTR1494del6* polymorphism and reduced risk of HCC. Although *in vitro* study showed reduced luciferase activity and mRNA levels related to *TYMS3′UTR1494del6*,[20] the *in vivo* function of this genetic polymorphism is unknown. Individuals possessing the *TYMS3′UTR1494* homozygous 6-bp deletion polymorphism showed higher circulatory folate and lower homocysteine levels than those with the homozygous 6-bp insertion polymorphism.[21] Epidemiological studies found inconsistent associations between the *TYMS3′UTR1494del6* polymorphism and risk of various diseases. Several studies reported that individuals possessing the −6/−6 genotype are at reduced risk of spina bifida (a folate-deficiency related birth defect),[44] lung cancer,[45] and gastric cancer.[46] Other studies reported that the homozygous deletion polymorphism was associated with increased risk of colorectal adenoma,[47] non-Hodgkin lymphoma,[48] and breast cancer.[49] Our results suggest that the 6-bp deletion polymorphism may have a protective effect on the development of HCC. The protective effect concurs with its conversion of dUMP to dTMP, especially under the condition of elevated 5,10-methylenetetrahydrofolate resulting from the *MTHFR* genetic mutation. The *TYMS3′UTR144del* allele has been found to be in linkage disequilibrium with the *TYMSER*3R* polymorphism; the latter is associated with high transcription and enzyme activity.[50] Therefore, the protective effect of the 6-bp deletion allele also may be reflective of other functional polymorphisms in the *TYMS* gene.

The current study had several limitations. The lack of dietary folate assessment did not allow us to examine the potential interaction effect between the *MTHFR/TYMS* genotypes and folate on risk of HCC. Another limitation is the lack of measurement on the *TYMSER*2R/3R* genotype. However, several studies that examined both the *TYMSER*2R/3R* and *TYMS3′ UTR* genetic polymorphisms found only the latter to be inversely associated with disease risk.[44-46] Because of the rapidly fatal nature of HCC, a large proportion (approximately 80% in Los Angeles) of HCC patients were not included in the study. Thus, the observed *MTHFR/ TYMS* genotype-HCC risk association would be biased if these genotypes had an impact on the patients' survival after HCC diagnosis.

Initially, we had hypothesized that MTHFR genetic variants with reduced activity might be linked to increased risk of HCC (as opposed to decreased risk that is being observed in the current study). This is based on the recent report that the *MTHFR C677T* polymorphism is associated with steatosis and fibrosis in chronic hepatitis C patients.[51] Reduced MTHFR activity can reduce the SAMe pool (especially if folate is deficient), and we have accumulating evidence especially in animal models that chronic hepatic SAMe deficiency can predispose to HCC.[22] Reduced SAMe availability can lead to global DNA hypomethylation, which occurs in HCC.[52] Although global DNA methylation is reduced, hypermethylation of growth suppressive genes such as p16 and p14 in promoter regions commonly occur.[53] Interestingly, 1 animal study showed progressive exon-specific hypomethylation of p53 in rats fed a lipotrope-deficient diet (deficient in folate, B_{12}, methionine, and choline), followed by rebound hypermethylation at a later time when neoplastic foci became evident.[12] This suggests that

global hypomethylation may trigger hypermethylation of specific genes. DNA hypomethylation results from reduced MTHFR activity also can lead to increased expression of oncogenes such as c-myc and c-N-ras, increased DNA strand breakage, and impairment in DNA repair.[12] However, reduced MTHFR activity would channel folate cofactors to DNA synthesis and repair. This would prevent incorporation of uracil into DNA, which can lead to DNA strand breakage.[54] A balance between the 2 critical pathways (methylation and DNA synthesis and repair) must be reached so that 1 does not compromise the other. The tipping point of this balance may be different at different stages of the oncogenesis process. Supporting this is the observation that folate deficiency predisposes to colon cancer, but once preneoplastic colon lesions are present, folate supplementation actually accelerates colon cancer transformation.[55] Folate supplementation may further enhance DNA synthesis in the already transformed cell. What is critical for the different stages of HCC development remains to be defined, including the role of dietary folate. The results of this study point to the importance of these pathways in determining the susceptibility to HCC and encourages additional studies to further define their influence in the different stages of hepatocarcinogenesis.

In summary, the current study shows protective effects of the *MTHFR677 TT, MTHFR1298 CC*, and the *TYMS3'UTR1494 -6/-6* genotypes on risk of HCC. One possible mechanistic pathway that can explain the protective effects of the 2 genes on HCC is the 1-carbon metabolism pathway favoring the conversion of dUMP to dTMP.

Acknowledgements

Supported in part by grants R35 CA53890 and R01 CA80205 (PI, Mimi C. Yu). In addition, Jian-Min Yuan was supported in part by Grants R01 CA43092 and R01 CA98497. Shelly C. Lu and José M Mato were supported in part by Grants AA12677, AA13847, and AT1576. Shelly Lu was also supported by DK51719. All grants were from the United States National Institutes of Health. José M. Mato was supported in part by Grants from PN I+D SAF 2005-00855 from the Spanish Ministry of Science, CIBER from the Spanish Ministry of Health, HEPADIP EULSHM-CT-2005 from the European Union, and ETORTEK 2005 from the Basque Department of Industry.

Appendix A

Primers and probes used for genotyping the *MTHFR* and *TYMS* gene polymorphisms

	rs number*	Primers (5′ - > 3′ orientation)*	MGB Probe (5′ - > 3′ orientation)	Ta
MTHFR677C→T	rs1801133	GACCTGAAGCACTTGAAGGAGAAG (f) CACAAAGCGGAAGAATGTGTCA(r)	ATGAAATCGACTCCCGC (6-FAM probe) TGAAATCGGCTCCCGC (VIC probe)	64° C
MTHFR1298A→C	rs1801131	GAGCAAGTCCCCCAAGGA (f) TTTGTGACCATTCCGGTTTG (r)	AAGACACTTTCTTCACTG (6-FAM probe) AGACACTTGCTTCACTG (VIC probe)	60° C
TYMS3′ UTR1949del6	rs16430	GGAGCTGAGTAACACCATCGATC (f) CGTGGACGAATGCAGAACA (r)	AAACAACTATAAAGTTCATAAC (6-FAM probe) CAACTATAACTTTAAAGTTCATAA (VIC probe)	63° C

* rs denotes for reference single nucleotide polymorphism; (f) forward primer, (r) reverse primer.

References

1. Ferlay, J.; Bray, F.; Pisani, P.; Parkin, DM. GLOBOCAN 2002. IARC CancerBase No 5, version 2.0 ed. Lyon: IARC Press; 2004. Cancer Incidence, Mortality and Prevalence Worldwide.
2. De Carlis L, Giacomoni A, Pirotta V, Lauterio A, Slim AO, Sammartino C, et al. Surgical treatment of hepatocellular cancer in the era of hepatic transplantation. J Am Coll Surg 2003;196:887–897. [PubMed: 12788425]
3. Yeh FS, Yu MC, Mo CC, Luo S, Tong MJ, Henderson BE. Hepatitis B virus, aflatoxins, and hepatocellular carcinoma in southern Guangxi, China. Cancer Res 1989;49:2506–2509. [PubMed: 2539905]

4. Kew MC, Yu MC, Kedda MA, Coppin A, Sarkin A, Hodkinson J. The relative roles of hepatitis B and C viruses in the etiology of hepatocellular carcinoma in southern African blacks. Gastroenterology 1997;112:184–187. [PubMed: 8978357]
5. Donato F, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. Int J Cancer 1998;75:347–354. [PubMed: 9455792]
6. Yu MC, Yuan JM. Environmental factors and risk for hepatocellular carcinoma. Gastroenterology 2004;127:S72–S78. [PubMed: 15508106]
7. Caldwell SH, Crespo DM, Kang HS, Al-Osaimi AM. Obesity and hepatocellular carcinoma. Gastroenterology 2004;127:S97–S103. [PubMed: 15508109]
8. Yuan JM, Govindarajan S, Arakawa K, Yu MC. Synergism of alcohol, diabetes, and viral hepatitis on the risk of hepatocellular carcinoma in blacks and whites in the U.S. Cancer 2004;101:1009–1017. [PubMed: 15329910]
9. Mikol YB, Hoover KL, Creasia D, Poirier LA. Hepatocarcinogenesis in rats fed methyl-deficient, amino acid-defined diets. Carcinogenesis 1983;4:1619–1629. [PubMed: 6317218]
10. Nakae D, Yoshiji H, Mizumoto Y, Horiguchi K, Shiraiwa K, Tamura K, et al. High incidence of hepatocellular carcinomas induced by a choline deficient L-amino acid defined diet in rats. Cancer Res 1992;52:5042–5045. [PubMed: 1516060]
11. Pogribny IP, Basnakian AG, Miller BJ, Lopatina NG, Poirier LA, James SJ. Breaks in genomic DNA and within the p53 gene are associated with hypomethylation in livers of folate/methyl-deficient rats. Cancer Res 1995;55:1894–1901. [PubMed: 7794383]
12. Pogribny IP, Muskhelishvili L, Miller BJ, James SJ. Presence and consequence of uracil in preneoplastic DNA from folate/methyl-deficient rats. Carcinogenesis 1997;18:2071–2076. [PubMed: 9395204]
13. Kim YI, Pogribny IP, Basnakian AG, Miller JW, Selhub J, James SJ, et al. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. Am J Clin Nutr 1997;65:46–52. [PubMed: 8988912]
14. Hori T, Ayusawa D, Shimizu K, Koyama H, Seno T. Chromosome breakage induced by thymidylate stress in thymidylate synthase-negative mutants of mouse FM3A cells. Cancer Res 1984;44:703–709. [PubMed: 6692373]
15. Hori T, Ayusawa D, Glover TW, Seno T. Expression of fragile site on the human X chromosome in somatic cell hybrids between human fragile X cells and thymidylate synthase-negative mouse mutant cells. Jpn J Cancer Res 1985;76:977–983. [PubMed: 3935628]
16. Kaneda S, Takeishi K, Ayusawa D, Shimizu K, Seno T, Altman S. Role in translation of a triple tandemly repeated sequence in the 5′-untranslated region of human thymidylate synthase mRNA. Nucl Acids Res 1987;15:1259–1270. [PubMed: 3029702]
17. Marsh S, Collie-Duguid ES, Li T, Liu X, McLeod HL. Ethnic variation in the thymidylate synthase enhancer region polymorphism among Caucasian and Asian populations. Genomics 1999;58:310–312. [PubMed: 10373329]
18. Kawakami K, Omura K, Kanehira E, Watanabe Y. Polymorphic tandem repeats in the thymidylate synthase gene is associated with its protein expression in human gastrointestinal cancers. Anticancer Res 1999;19:3249–3252. [PubMed: 10652619]
19. Ulrich CM, Bigler J, Velicer CM, Greene EA, Farin FM, Potter JD. Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. Cancer Epidemiol Biomarkers Prev 2000;9:1381–1385. [PubMed: 11142426]
20. Mandola MV, Stoehlmacher J, Zhang W, Groshen S, Yu MC, Iqbal S, et al. A 6 bp polymorphism in the thymidylate synthase gene causes message instability and is associated with decreased intratumoral TS mRNA levels. Pharmacogenetics 2004;14:319–327. [PubMed: 15115918]
21. Kealey C, Brown KS, Woodside JV, Young I, Murray L, Boreham CA, et al. A common insertion/deletion polymorphism of the thymidylate synthase (TYMS) gene is a determinant of red blood cell folate and homocysteine concentrations. Hum Genet 2005;116:347–353. [PubMed: 15682292]
22. Lu SC, Mato JM. Role of methionine adenosyltransferase and S-adenosylmethionine in alcohol-associated liver cancer. Alcohol 2005;35:227–234. [PubMed: 16054984]

23. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–113. [PubMed: 7647779]
24. van der Put NM, Gabreels F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 1998;62:1044–1051. [PubMed: 9545395]
25. Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). Thromb Haemost 1997;78:523–526. [PubMed: 9198208]
26. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998;64:169–172. [PubMed: 9719624]
27. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A 1997;94:3290–3295. [PubMed: 9096386]
28. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol 2004;159:423–443. [PubMed: 14977639]
29. Nieters A, Yuan JM, Sun CL, Zhang ZQ, Stoehlmacher J, Govindarajan S, et al. Effect of cytokine genotypes on the hepatitis B virus-hepatocellular carcinoma association. Cancer 2005;103:740–748. [PubMed: 15643599]
30. Lee LG, Connell CR, Bloch W. Allelic discrimination by nick-translation PCR with fluorogenic probes. Nucl Acids Res 1993;21:3761–3766. [PubMed: 8367293]
31. Breslow, NE.; Day, NE. Statistical methods in cancer research Volume 1 The analysis of case-control studies. Lyon: IARC Scientific Publication; 1980. p. 192-279.
32. Slatkin M, Excoffier L. Testing for linkage disequilibrium in genotypic data using the Expectation-Maximization algorithm. Heredity 1996;76:377–383. [PubMed: 8626222]
33. Ardlie KG, Kruglyak L, Seielstad M. Patterns of linkage disequilibrium in the human genome. Nat Rev Genet 2002;3:299–309. [PubMed: 11967554]
34. Kleinbaum, DG.; Kupper, LL.; Muller, KE.; Nizam, A. Applied regression analysis and other multivariable methods. Pacific Grove, California: Duxbury Press; 1998.
35. Chango A, Boisson F, Barbe F, Quilliot D, Droesch S, Pfister M, et al. The effect of 677C→T and 1298A→C mutations on plasma homocysteine and 5,10-methylenetetrahydrofolate reductase activity in healthy subjects. Br J Nutr 2000;83:593–596. [PubMed: 10911766]
36. Yin G, Kono S, Toyomura K, Hagiwara T, Nagano J, Mizoue T, et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and colorectal cancer: the Fukuoka Colorectal Cancer Study. Cancer Sci 2004;95:908–913. [PubMed: 15546509]
37. Saffroy R, Pham P, Chiappini F, Gross-Goupil M, Castera L, Azoulay D, et al. The MTHFR 677C > T polymorphism is associated with an increased risk of hepatocellular carcinoma in patients with alcoholic cirrhosis. Carcinogenesis 2004;25:1443–1448. [PubMed: 15033905]
38. Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997;57:1098–1102. [PubMed: 9067278]
39. Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM, et al. Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. Epidemiology 2001;12:420–428. [PubMed: 11416780]
40. Yang CX, Matsuo K, Ito H, Shinoda M, Hatooka S, Hirose K, et al. Gene-environment interactions between alcohol drinking and the MTHFR C677T polymorphism impact on esophageal cancer risk: results of a case-control study in Japan. Carcinogenesis 2005;26:1285–1290. [PubMed: 15790587]
41. Hidiroglou N, Camilo ME, Beckenhauer HC, Tuma DJ, Barak AJ, Nixon PF, et al. Effect of chronic alcohol ingestion on hepatic folate distribution in the rat. Biochem Pharmacol 1994;47:1561–1566. [PubMed: 8185668]
42. Chiuve SE, Giovannucci EL, Hankinson SE, Hunter DJ, Stampfer MJ, Willett WC, et al. Alcohol intake and methylenetetrahydrofolate reductase polymorphism modify the relation of folate intake to plasma homocysteine. Am J Clin Nutr 2005;82:155–162. [PubMed: 16002814]

43. Matsuo K, Ito H, Wakai K, Hirose K, Saito T, Suzuki T, et al. One-carbon metabolism related gene polymorphisms interact with alcohol drinking to influence the risk of colorectal cancer in Japan. Carcinogenesis 2005;26:2164–2171. [PubMed: 16051637]

44. Volcik KA, Shaw GM, Zhu H, Lammer EJ, Laurent C, Finnell RH. Associations between polymorphisms within the thymidylate synthase gene and spina bifida. Birth Defects Research 2003;67:924–928. [PubMed: 14745930]

45. Shi Q, Zhang Z, Li G, Pillow PC, Hernandez LM, Spitz MR, et al. Polymorphisms of methionine synthase and methionine synthase reductase and risk of lung cancer: a case-control analysis. Pharmacogenetics & Genomics 2005;15:547–555. [PubMed: 16006998]

46. Zhang Z, Xu Y, Zhou J, Wang X, Wang L, Hu X, et al. Polymorphisms of thymidylate synthase in the 5′- and 3′-untranslated regions associated with risk of gastric cancer in South China: a case-control analysis. Carcinogenesis 2005;26:1764–1769. [PubMed: 15930032]

47. Ulrich CM, Bigler J, Bostick R, Fosdick L, Potter JD. Thymidylate synthase promoter polymorphism, interaction with folate intake, and risk of colorectal adenomas. Cancer Res 2002;62:3361–3364. [PubMed: 12067974]

48. Lightfoot TJ, Skibola CF, Willett EV, Skibola DR, Allan JM, Coppede F, et al. Risk of non-Hodgkin lymphoma associated with polymorphisms in folate-metabolizing genes. Cancer Epidemiol Biomarkers Prev 2005;14:2999–3003. [PubMed: 16365025]

49. Zhai X, Gao J, Hu Z, Tang J, Qin J, Wang S, et al. Polymorphisms in thymidylate synthase gene and susceptibility to breast cancer in a Chinese population: a case-control analysis. BMC Cancer 2006;6:138. [PubMed: 16723031]

50. Morganti M, Ciantelli M, Giglioni B, Putignano AL, Nobili S, Papi L, et al. Relationships between promoter polymorphisms in the thymidylate synthase gene and mRNA levels in colorectal cancers. Eur J Cancer 2005;41:2176–2183. [PubMed: 16182121]

51. Adinolfi LE, Ingrosso D, Cesaro G, Cimmino A, D'Anto M, Capasso R, et al. Hyperhomocysteinemia and the MTHFR C677T polymorphism promote steatosis and fibrosis in chronic hepatitis C patients. Hepatology 2005;41:995–1003. [PubMed: 15834927]

52. Lin CH, Hsieh SY, Sheen IS, Lee WC, Chen TC, Shyu WC, et al. Genome-wide hypomethylation in hepatocellular carcinogenesis. Cancer Res 2001;61:4238–4243. [PubMed: 11358850]

53. Tal-Kremer, S.; Day, CP.; Reeves, HL. Genetic basis of hepatocellular cancer. In: Ali, S.; Friedman, SL.; Mann, DA., editors. Liver diseases biochemical mechanisms and new therapeutic insights. 2006. Enfield, New Hampshire: Science Publishers; p. 273-308.

54. Dianov GL, Timchenko TV, Sinitsina OI, Kuzminov AV, Medvedev OA, Salganik RI. Repair of uracil residues closely spaced on the opposite strands of plasmid DNA results in double-strand break and deletion formation. Mol Gen Genet 1991;225:448–452. [PubMed: 2017139]

55. Kim YI. Role of folate in colon cancer development and progression. J Nutr 2003;133:3731S–3739S. [PubMed: 14608107]

Abbreviations

2R
: double repeat

3R
: triple repeat

bp
: base pair

dTMP
: deoxythimidine monophosphate

dUTP
: deoxyuridine monophosphate

HBV
: hepatitis B virus

HCC
: hepatocellular carcinoma

HCV
: hepatits C virus

MTHFR
: methylenetetrahydrofolate reductase

SAMe
: S-adenosylmethionine

TYMS
: thymidylate synthase

TYMSER
: enhancer region of the TYMS gene

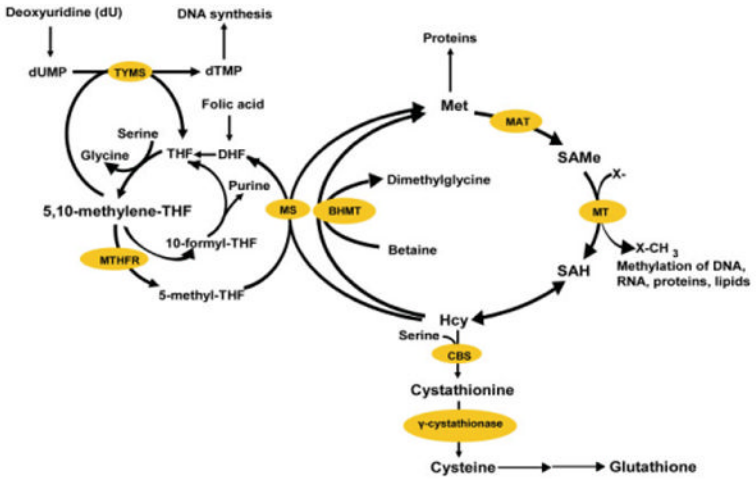

Fig. 1.

Schematic representation of 1-carbon metabolism. BHMT, betaine homocysteine methyltransferase; CBS, cystathionine *β*-synthase; DHF, dihydrofolate, dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; Hcy, homocysteine; MAT, methinione adenosyltransferase; Met, methinione; MS, methionine synthase; MT, methyl transferase; MTA, methylthioadenosine; MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenosylhomocysteine; SAMe, S-adenosylmethionine; THF, tetrahydrofolate; TYMS, thymidylate synthase.

Table 1

Distributions of Demographic Characteristics in Hepatocellular Carcinoma (HCC) Patients and Control Subjects by Study Location

Demographics	Los Angeles, California		Guangxi, China	
	HCC Patients (%) (n = 118)	Control Subjects (%) (n = 209)	HCC Patients (%) (n = 247)	Control Subjects (%) (n = 248)
Age (years)				
<40	3.4	3.4	18.6	18.6
40-<50	11.0	18.2	32.8	32.7
50-<60	27.1	24.4	33.2	28.2
60-<70	39.8	39.2	15.4	20.6
≥70	18.6	14.8	0.0	0.0
2-sided P	*0.50*		*0.42*	
Sex				
Males	68.6	61.2	87.8	88.3
Females	31.4	38.8	12.2	11.7
2-sided P	*0.18*		*0.88*	
Race/ethnicity				
Non-Hispanic white Americans	58.5	79.9	—	—
Hispanic Americans	33.9	15.3	—	—
African Americans	7.6	4.8	—	—
Chinese–Han ethnicity	—	—	79.8	80.2
Chinese–Zhuang/Yao ethnicity	—	—	20.2	19.8
2-sided P	*0.0001*		*0.60*	
Level of education				
Below high school	19.5	6.2	46.1	41.9
High school graduates	27.1	22.5	31.6	35.1
Some college/occupational school	33.9	33.5	15.4	13.7
College graduates or above	19.5	37.8	6.9	9.3
2-sided P	*<0.0001*		*0.56*	

Table 2

Distributions of Risk Factors for Hepatocellular Carcinoma (HCC) in HCC Patients and Control Subjects

Risk Factors and Levels*	Los Angeles, California		Guangxi, China		Total	
	Ca/Co†	OR (95% CI)‡	Ca/Co†	OR (95% CI)‡	Ca/Co†	OR (95% CI)‡
Cigarette smoking						
Non- or long ex-smokers	70/150	1.00	140/163	1.00	210/313	1.00
Current or recent ex-smokers	48/59	1.69 (1.02-2.77)	107/85	1.53 (1.04-2.23)	155/144	1.52 (1.13-2.04)
<20 cigarettes/day	14/25	1.00 (0.47-2.13)	31/29	1.29 (0.74-2.27)	45/54	1.19 (0.76-1.85)
≥ 20 cigarettes/day	34/34	2.24 (1.25-4.01)	76/56	1.65 (1.08-2.54)	110/90	1.72 (1.22-2.41)
2-sided P for trend		*0.01*		*0.02*		*0.002*
No. alcoholic drinks/day						
Nondrinkers	34/66	1.00	158/208	1.00	192/274	1.00
<2	19/79	0.44 (0.22-0.87)	37/19	2.71 (1.49-4.95)	56/98	1.29 (0.85-1.96)
2-<4	15/39	0.69 (0.31-1.52)	30/14	2.99 (1.52-5.87)	45/53	1.83 (1.13-2.96)
≥4	50/25	3.57 (1.68-7.58)	22/7	4.38 (1.81-10.58)	72/32	6.03 (3.47-10.45)
2-sided P for trend		*0.0004*		*<0.0001*		*<0.0001*
Hepatitis B serology						
Negative	85/185	1.00	43/213	1.00	128/398	1.00
Anti-HBc positive only	30/24	2.27 (1.21-4.26)	…	…	…	…
HBsAg positive	3/0	(1.24-§§)	204/35	42.1 (23.89-74.29)	207/35	37.36 (22.31-62.58)
2-sided P for trend		*0.01*		*<0.0001*		*<0.0001*
Hepatitis C serology						
Anti-HCV negative	61/208	1.00	238/245	1.00	299/453	1.00
Anti-HCV positive	57/1	173.5 (23.4-1284.3)	9/3	3.12 (0.83-11.69)	66/4	44.15 (15.50-125.76)
2-sided P for trend		*<0.0001*		*0.09*		*<0.0001*
Hepatitis B/C serology						
Both negative	58/208	1.00	43/211	1.00	101/419	1.00
Either positive¶	60/1	193.5 (26.1-1432.1)	204/37	38.21 (21.97-66.45)	264/38	42.18 (26.22-67.88)
2-sided P for trend		*<0.0001*		*<0.0001*		*<0.0001*

*Anti-HBC, antibodies to hepatitis B core antigen; HBsAg, hepatitis B surface antigen; anti-HCV, antibodies to hepatitis C virus.

†Ca/Co, number of HCC patients/number of control subjects.

‡Adjusted for age, sex, race/ethnicity, and study location (for total subjects only); OR, odds ratio; CI, confidence interval.

§There were no control subjects in this category. Therefore, no finite estimate for the odds ratio or its upper 95% confidence interval could be calculated.

¶Positive for either hepatitis B surface antigen or antibodies to hepatitis C virus.

Table 3

The Genotypic and Allelic Frequencies of the *MTHFR* and *TYMS3'UTR* Polymorphism Among Control Subjects by Race/Ethnicity

Genetic Polymorphism	Los Angeles, California		Guangxi, China		2-sided *P**
	Non-Hispanic Whites (n = 167)	Blacks/Hispanics (n = 42)	Han Chinese (n = 199)	Zhuang/Yao Chinese (n = 49)	
MTHFR677					
CC	0.38	0.40	0.65	0.55	<0.0001
CT	0.47	0.48	0.28	0.37	
TT	0.15	0.12	0.07	0.08	
C	0.61	0.64	0.79	0.73	
T	0.39	0.36	0.21	0.27	
2-sided *P* for HW†	*0.98*	*0.81*	*0.03*	*0.69*	
2-sided P‡	*0.87*		*0.44*		
MTHFR1298					
AA	0.48	0.57	0.51	0.69	0.20
AC	0.43	0.31	0.40	0.24	
CC	0.09	0.12	0.09	0.06	
A	0.69	0.73	0.71	0.82	
C	0.31	0.27	0.29	0.18	
2-sided *P*†	*0.83*	*0.15*	*0.63*	*0.20*	
2-sided *P*‡	*0.35*		*0.07*		
TYMS3'UTR1494					
+6/+6	0.41	0.38	0.06	0.04	<0.0001
+6/−6	0.43	0.55	0.37	0.41	
−6/−6	0.16	0.07	0.57	0.55	
+6	0.63	0.65	0.24	0.24	
−6	0.37	0.35	0.76	0.76	
2-sided *P*†	*0.32*	*0.17*	*0.95*	*0.47*	
2-sided P‡	*0.25*		*0.79*		

* Test for differences in genotypic frequencies between the 4 racial/ethnic groups.

† Test for the Hardy-Weinberg linkage equilibrium within each racial/ethnic group.

‡ Test for differences in genotypic frequencies between the 2 racial/ethnic groups within a given study location.

Table 4

Genetic Polymorphisms in the Methylenetetrahydrofolate Reductase (*MTHFR*) and Thymidylate Synthase (*TYMS*) Genes in Relation to Risk of Hepatocellular Carcinoma (HCC)

Genotype	Los Angeles, California		Guangxi, China		Total	
	Ca/Co*	OR (95% CI)†	Ca/Co*	OR (95% CI)†	Ca/Co*	OR (95% C2)†
MTHFR677						
CC	53/80	1.00	159/156	1.00	212/236	1.00
CT	51/99	0.85 (0.47-1.55)	71/74	0.94 (0.53-1.65)	122/173	0.91 (0.61-1.35)
TT	14/30	0.72 (0.29-1.78)	17/18	0.60 (0.22-1.62)	31/48	0.73 (0.38-1.43)
2-sided P for trend		*0.44*		*0.39*		*0.36*
MTHFR1298						
AA	65/104	1.00	136/136	1.00	201/240	1.00
AC	44/85	1.09 (0.60-1.99)	101/91	0.91 (0.54-1.55)	145/176	1.00 (0.68-1.47)
CC	9/20	0.54 (0.19-1.55)	10/21	0.60 (0.20-1.84)	19/41	0.50 (0.23-1.05)
2-sided P for trend		*0.50*		*0.43*		*0.22*
Sum of *MTHFR* variant alleles						
0	23/26	1.00	77/65	1.00	100/91	1.00
1	49/82	1.07 (0.47-2.46)	114/123	0.82 (0.46-1.48)	163/205	0.87 (0.55-1.37)
2	46/101	0.64 (0.28-1.49)	56/60	0.61 (0.30-1.23)	102/161	0.62 (0.37-1.03)
2-sided P for trend		*0.17*		*0.17*		*0.06*
TYMS3'UTR1494						
+6/+6	60/85	1.00	16/14	1.00	76/99	1.00
+6/−6	44/95	0.50 (0.27-0.92)	99/93	0.68 (0.24-1.90)	143/188	0.51 (0.30-0.85)
−6/−6	14/29	0.46 (0.17-1.22)	132/141	0.74 (0.27-2.04)	146/170	0.53 (0.30-0.95)
2-sided P for trend		*0.03*		*0.87*		*0.07*
Sum of *MTHFR* and *TYMS* variant alleles						
0-1	49/55	1.00	42/41	1.00	91/96	1.00
2	41/79	0.44 (0.22-0.86)	96/76	1.02 (0.49-2.12)	137/155	0.68 (0.42-1.10)
3	22/60	0.31 (0.14-0.68)	80/94	0.74 (0.35-1.54)	102/154	0.47 (0.28-0.78)
4	6/15	0.38 (0.10-1.46)	29/37	0.62 (0.25-1.55)	35/52	0.46 (0.23-0.93)
2-sided P for trend		*0.005*		*0.18*		*0.003*

*Ca/Co, number of HCC patients/number of control subjects.

†Adjusted for age, sex, race/ethnicity, study location (for total subjects only), level of education, number of cigarettes smoked per day, number of alcoholic drinks per day, and hepatitis B/C serology; OR, odds ratio; CI, confidence interval.

Table 5

The Joint Genotypes of the *MTHFR677* and *MTHFR1298* Polymorphisms and Risk of Hepatocellular Carcinoma

MTHFR677	MTHFR1298					
	AA		AC		CC	
	Ca/Co*	OR (95% CI)†	Ca/Co*	OR (95% CI)†	Ca/Co*	OR (95% CI)†
CC	100/91	1.00	93/104	0.93 (0.55-1.56)	19/41	0.42 (0.19-0.94)
CT	70/101	0.81 (0.47-1.40)	52/72	0.74 (0.40-1.37)	0/0	—
TT	31/48	0.63 (0.31-1.29)	0/0	—	0/0	—

*Ca/Co, number of HCC patients/number of control subjects.

†Adjusted for age, sex, race/ethnicity, study location, level of education, number of cigarettes smoked per day, number of alcoholic drinks per day, and hepatitis B/C serology.

Abbreviations: OR, odds ratio; CI, confidence interval.