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Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms and the risk of primary Hepatocellular Carcinoma (HCC) in a Chinese population

Li-Na Mu,
Department of Epidemiology, Fudan University School of Public, Health, Shanghai, China

Department of Epidemiology and Public Health, School of, Medicine, Yale University, 60 College Street, LEPH 400, New Haven, CT 06520-8034, USA

Wei Cao,
Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA 90095, USA

Zuo-Feng Zhang,
Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA 90095, USA

Lin Cai,
Department of Epidemiology, Fujian Medical University, Fuzhou, Fujian, China

Qing-Wu Jiang,
Department of Epidemiology, Fudan University School of Public, Health, Shanghai, China

Nai-Chieh You,
Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA 90095, USA

Binh Yang Goldstein,
Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA 90095, USA

Guo-Rong Wei,
Department of Epidemiology, Fudan University School of Public, Health, Shanghai, China

Chuan-Wei Chen,
Department of Epidemiology, Fudan University School of Public, Health, Shanghai, China

Qing-Yi Lu,
UCLA Center for Human Nutrition, School of Medicine, Los Angeles, CA 90095, USA

Xue-Fu Zhou,
Taixing Center of Disease Control & Prevention, Taixing, Jiangsu, China

Bao-Guo Ding,
Taixing Center of Disease Control & Prevention, Taixing, Jiangsu, China

Jun Chang, and
Taixing Center of Disease Control & Prevention, Taixing, Jiangsu, China

Correspondence to: Li-Na Mu, lina.Mu@yale.edu.

Shun-Zhang Yu
Department of Epidemiology, Fudan University School of Public, Health, Shanghai, China

Li-Na Mu: lina.Mu@yale.edu

Abstract

Objectives—Methylenetetrahydrofolate reductase (MTHFR), which is expressed in the liver, may be involved in both DNA methylation and DNA synthesis. It is also indicated as a potential risk factor of liver cancer in patients with chronic liver disease. To date, no study has been conducted on MTHFR and hepatocellular carcinoma (HCC) using a population-based design. The objective of this study was to evaluate the effects of polymorphisms of the MTHFR gene on the risk of primary liver cancer and their possible effect modifications on various environmental risk factors.

Methods—A population-based case–control study was conducted in Taixing, China. MTHFR C677T and A1298C were assayed by PCR-RFLP techniques.

Results—The frequency of MTHFR 677 C/C wild homo-zygotes genotype was 25.8% in cases, which was lower than that in controls (34.5%). The adjusted odds ratios (ORs) for the MTHFR 677 C/T and T/T genotype were 1.66(95% CI: 1.06–2.61), 1.21(95% CI: 0.65–2.28) respectively when compared with the MTHFR 677 C/C genotype. Subjects carrying any T genotype have the increased risk of 1.55(95% CI: 1.01–2.40) for development of primary hepatocellular carcinoma. A high degree of linkage disequilibrium was observed between the C677T and A1298C polymorphisms, with the D' of 0.887 and $p < 0.01$. The MTHFR 677 any T genotype was suggested to have potentially more than multiplicative interactions with raw water drinking with *p*-value for adjusted interaction of 0.03.

Conclusion—We observed that the MTHFR 677 C/T genotype was associated with an increased risk of primary liver cancer in a Chinese population. The polymorphism of MTHFR 677 might modify the effects of raw water drinking on the risk of primary hepatocellular carcinoma.

Keywords

MTHFR (5, 10-methylenetetralydrofolate reductase); Genetic polymorphism; Primary liver cancer; Case–control study; Effect modification

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer (*n* = 564,336) and the third most frequent cause of cancer deaths (*n* = 548,554) in the world in 2000. It is largely a problem in developing countries, where 81% of the world's total cases occur. Areas of highest risk include West and Central Africa, Eastern and South-Eastern Asia, and Melanesia [1]. According to Globocan 2000, 308,437 new cases of HCC were estimated to have occurred in China each year, which accounts for 54.7% of all incident cancer cases in the world. HCC is the second leading cause of cancer deaths in China, with 301,536 deaths, which accounts for 55% of all liver cancer deaths in the world. The disease is considered the third most common cause of cancers in the study area (Taixing city, China), where the crude

and adjusted incidence rates of HCC were 56/100,000 and 31.3/100,000, respectively, in 2000.

Compared with the well-established associations between environmental risk factors and development of HCC, genetic susceptibility factors of HCC are not as extensively studied or understood.

The MTHFR (5,10-methylenetetralydrofolate reductase) enzyme is part of a complex metabolic entity involved in both the generation of S-adenosylmethionine, a universal methyl-group donor, and DNA synthesis. Single nucleotide polymorphisms (SNPs) in the MTHFR gene have been identified. A C-to-T transition at nucleotide 677 (C677T) in exon four results in an alanine to valine exchange and affects the catalytic domain of the enzyme, which leads to reduced enzyme activity. The enzyme activity levels are approximately 70% lower than the common form, and individuals who are homozygous and heterozygous for this polymorphism have an increased amount of homocysteine [2, 3]. Another common variant of the MTHFR gene is an A-to-C transversion at position 1298 (A1298C) in exon seven, which causes a glutamine to alanine exchange at position 429; this polymorphism influences specific activity of the enzyme to a lesser extent than the MTHFR C677T polymorphism [4].

Associations between polymorphisms of the MTHFR gene and the risks of other cancers sites have been examined in several studies; however, the results are inconsistent. Genotypes with the MTHFR 677T allele have been related to protective effects on colon/rectum cancer [5–9], acute lymphocytic leukemia [10–12], and increased cancer risk of the bladder [13], cervix [14], breast [15, 16], esophagus [17], and stomach [18]. However, only one epidemiological study estimated the relationship between MTHFR polymorphisms and liver cancer among patients with alcoholic cirrhosis and suggested that the MTHFR 677CC genotype increased the risk of developing HCC among patients with high alcohol consumption [19]. No studies, however, have examined the relationship between the MTHFR A1298C polymorphism and risk for liver cancers.

Besides, possible interaction between major risk factors of HCC and MTHFR SNPs were intriguing. First, the liver is the main site for the storage and the metabolic handing of all vitamins (vitamin B12 and B6, and folates, which function as key cofactors for metabolic activity of enzymes involving homocysteine clearance [20]). Meanwhile, the liver is also a well-known key organ for the metabolic processing of carcinogens. Damage to the liver caused by aflatoxin B1, smoke toxins, ethanol consumption, as well as inflammatory reactions of HBV/HCV infection, may lead to the alternation of folate metabolism, which may interact with MTHFR enzyme activity and play an important role in the development of HCC. Secondly, it was also reported that cigarette smoking and alcohol consumption were both inversely associated with plasma folate concentration. Alcohol or smoke toxins may directly damage liver cells, leading to liver cancer and both exposures may go through the folic acid (one carbon metabolic) pathway, which is associated with MTHFR. It is worthwhile to look at the possible interaction between alcohol drinking, cigarette smoking and MTHFR. Thirdly, MTHFR C667T has also been associated in several reports with an increased risk of invasive cervical cancer and premalignant lesions [21], which also indicate

a possible role of MTHFR in viral infection associated cancers. Further, MTHFR C677T polymorphism is associated with hype-rhomocysteinemia among patients with chronic hepatitis C infection [22], which may accelerate the progression of liver fibrosis in CHC and possibly lead to liver cancer. It would be interesting to evaluate the potential interaction between MTHFR and HCV infection. All these may indicate that the MTHFR may interact with environmental risk factors and play a role in the development of HCC.

The purposes of this population-based case–control study are to assess the associations between the MTHFR C677T and A1298C polymorphisms and the risk of HCC and to explore the potential modification of effects of hepatitis virus infections and other environmental risk factors by polymorphisms on HCC risk.

Materials and methods

Background

Taixing City (formerly Taixing County, prior to 1995) is located on the east bank of the Yangtze River in Jiangsu Province in southeast China. The population-based tumor registry is within the Division of Chronic Disease Prevention, Taixing City Center for Disease Prevention and Control (CDC). Taixing City has 23 townships (rural areas) and one central town (urban area). Each township or city has 10–12 villages (or resident blocks in the urban areas). Each village (or resident block) has one county doctor who is responsible for reporting new cancer cases and deaths to the disease prevention and control division of the district (or township) hospital, after which the information is reported by the district hospital to the Taixing City CDC's population-based tumor registry twice a month. The central town has a similar reporting system (resident blocks and town hospital). Taixing is one of the highest-risk areas of alimentary cancer in China. The incidence rate was 56/ 100,000 for HCC in 2000. The disease is the second leading cause of cancer deaths, following esophageal cancer.

Study population

A population-based case–control study was conducted in Taixing City, Jiangsu Province, China. Data collected included questionnaire data and blood samples for assaying molecular markers. Although the original study included three cancer sites (esophagus, stomach, and liver) and one common population-control group, the case group for this analysis only included patients with newly diagnosed primary HCC and population controls. The healthy population control group was randomly selected from the local population from which the cases were derived.

>Cases—Eligible cases were patients with pathologically or clinically confirmed diagnoses of primary HCC from 1 January 2000, to 30 June 2000, reported to the Taixing Tumor Registry at the Taixing CDC. During the study period, we intended to interview all incident cases with primary liver cancer that consented to participate in the study with the following restrictions: Patients must be newly diagnosed, aged 20 years or older, in stable medical condition as determined by their physicians, and willing to participate. The study was restricted to people living in Taixing for 10 or more years. In the six-month study

period, we recruited a total of 204 patients with primary HCC, which represents 57% of all new cases (n = 358) diagnosed in Taixing. Among these cases, all 204 patients completed the questionnaires, and 194 DNA samples were isolated. Five percent of cases (n = 10) had inadequate blood samples for DNA extraction.

Controls—Eligible controls were randomly selected, healthy individuals from the general population in Taixing City. Since the original study included three upper-GI cancers (stomach, liver, and esophagus), we used a common control group for all three cancer sites. We interviewed eligible controls during the study period with the following criteria: aged 20 years or older, in stable medical condition, and willing to participate. The study was restricted to people living in Taixing for 10 years or more. The control group was selected according to the frequency distribution of sex and age of all three-cancer cases interviewed from each village (or resident block in the city) where cancer cases originated. For each village (or resident block), a list was generated of residents within the same gender and age group, and random numbers were used to select healthy controls according to the control-to-case ratio of 2:3. When the control did not fit the criteria, or if he/she had refused to be interviewed, we recorded his/her basic demographic data and used the same selection process to choose another control. On average, 18–20 healthy controls were selected for each township (center town). A total of 464 controls were finally selected from the whole population of 1.28 million residents in the Taixing area. Due to the method of control selection, the age and sex distribution of controls were correspondent to all three-cancer sites and might not necessarily match the distribution of liver cancer cases.

Following the selected list, the interviewer located the controls, explained the study, interviewed them at their homes, and collected approximately 8 ml of blood. A total of 464 potential healthy controls were approached and 415 completed interviews (89.4%). Among the controls who had completed interviews, a total of 397 DNA samples were isolated from blood specimens. Four percent of interviewed controls did not have DNA samples for analysis due to no or insufficient collected blood samples. MTHFR C677T and A1298C genotypes were successfully assayed in 391 and 394 DNA samples, respectively.

Epidemiologic data collection

We interviewed cases and controls using a standard questionnaire. Our interviewers received rigorous training. Interviews were monitored by professional staff in the Division of Chronic Disease Prevention of the Taixing CDC. For cases, the interviews took place either in the hospital or at the study subjects' homes. All healthy control subjects were interviewed at their villages. We attempted to include all known and possible risk or protective factors that were considered important in the Chinese population using a standard questionnaire. The questionnaire included (1) demographic factors, including the subject's age, gender, residence, place of birth, education, annual income, blood type, and disease diagnostic information; (2) residence and drinking water history, including "raw water" intake history (Raw water drinking is defined as "drinking uncooked water." People who drank raw water more than four times per week were considered raw water drinkers.); (3) detailed dietary history, focusing specifically on the ingestion of moldy food (Exposure to moldy food was defined as individuals who had moldy food in home storage and ate it frequently in the last

10 years.); (4) detailed smoking history; (5) alcohol drinking habits; (6) tea drinking habits; (7) detailed information on disease history; (8) occupation history and related exposures; (9) family history of stomach cancer and other cancers; and (10) physical activities.

Laboratory assays

Antigens and Antibodies for Hepatitis viruses. The presence of HBsAg in serum was measured by enzyme-linked immunosorbant assay (ELISA) using kits from the Reagent Company of the Shanghai Hospital for Infectious Diseases (Shanghai, China). Anti-HCV IgG antibody was measured by ELISA using kits from Shanghai Huamei Biological Company (Shanghai, China). Both HBsAg and anti-HCV IgG were assayed according to the manufacturers' instructions.

PCR-analysis of gene polymorphisms. Genotyping was performed in the Molecular Epidemiology Laboratory at UCLA. All reagents were obtained from Promega Company (Madison, WI). PCR-RFLP analyses were modified from methods described previously [23, 24]. For the C677T polymorphism, C/C homozygotes have one (198 bp) fragment only; C/T heterozygotes have three fragments (198, 175, and 23bp); and T/T homozygotes have two (175 and 23 bp) fragments. For the A1298C polymorphism, A/A wild-type homozygotes have five fragments (56, 31, 30, 28, and 18 bp); A/C heterozygotes have six fragments (84, 56, 31, 30, 28, and 18 bp); and C/C homozygotes have four fragments (84, 31, 30, and 18 bp).

Definition of vegetable and fruit intake level

We assume that the ingestion of either fruits or vegetables has a similar effect on a person's odds of developing cancer. In studying these effects we assigned a score to each study subject according to his or her intake of fruits, and a second score based on vegetable intake. In both cases the classification process worked as follows: Subjects with a fruit and vegetable intake frequency lower than 50% were placed in the low-intake group and given a score of zero. Subjects with a fruit and vegetable intake frequency higher than 50%, but lower than 75% were placed in the middle intake group and given a score of one. Subjects with a fruit and vegetable intake frequency higher than 75% were placed in the high-intake group and given a score of two. We then assigned each subject a third score that consisted of the sum of the first two scores. According to the sum of scores calculated from fruit and vegetable intake, each subject fell into one of two groups. The low fruit and vegetable intake group had less than four points while the high fruit and vegetable intake group had more than four points."

Statistical analysis

All analyses were performed using SAS 8.0 software. We evaluated relationships between primary HCC and putative risk factors by crude and adjusted odds ratios (ORs) and their 95% CIs derived from an unconditional logistic regression model. Crude ORs and adjusted ORs were estimated for each independent variable. A logistic regression model was used to evaluate the multiplicative interaction effects. We adjusted for potential confounding factors including age (continuous), gender (male = 1, female = 0), education (categorical), and

HBsAg. To test linkage disequilibrium between MTHFR 677C>T and 1298A>C polymorphisms, D' was calculated by using the Haploview software.

Results

Table 1 shows the distribution of age, gender, education, and average income per capita among cases and controls. We observed a higher proportion of males among cases (77.94%) compared to controls (69.16%), and a higher proportion of younger individuals (<50 years old) among cases than controls ($p < 0.05$). Compared with cases, the levels of education were higher among controls. A borderline difference was observed for average income between cases and controls, with a higher proportion of HCC cases distributed in lower-income classes than controls.

Crude and adjusted ORs and 95% CIs of the potential environmental risk factors of HCC are presented in Table 2. HBsAg and anti-HCV markers for chronic infections of HBV and HCV were much more prevalent among cases than controls, with adjusted ORs of 5.14 (95% CI: 3.50–7.55) and 3.39 (95% CI: 1.43–8.03), respectively. Ingestion of moldy foods, a surrogate of AFB1 exposure, was associated with a moderate increase in the risk of HCC, with an adjusted OR of 2.25 (95% CI: 1.39–3.64). Raw water drinking was related to an increased risk of HCC and a dose-response association was shown (p for trend < 0.01). No obvious associations were observed between tobacco smoking and alcohol drinking and risk of HCC. Family history of liver cancer was related to a higher risk of HCC, with an adjusted OR of 2.99 (95% CI: 1.78– 5.01). In addition, high fruit and vegetable intake was found associated with decreased risk of HCC, with an adjusted OR of 0.58 (95% CI: 0.38–0.89).

The associations between MTHFR genetic susceptibility and HCC are shown in Table 3. Using the C/C genotype as the reference, the adjusted ORs were 1.66 (95% CI: 1.06– 2.61) and 1.21 (95% CI: 0.65–2.28) for the C/T and T/T genotypes, respectively. The adjusted OR for the any T genotype was 1.55 (95% CI: 1.01–2.40) when compared with the C/C genotype. Genotype frequencies of MTHFR A1298C were comparable between cases and controls. When comparing the A/C or C/C genotype with the A/A genotype, the adjusted OR was 1.02 (95% CI: 0.67–1.57). Using the MTHFR C677T any T genotype and A1298C any C genotype as risk genotypes, we evaluated the combined effect of different exposures on the risk of HCC. MTHFR 677 C/T or T/T genotype combined with MTHFR 1298 A/A genotype were associated with an increased risk of HCC, with the OR, 2.03 (95% CI: 1.12~3.68).

Furthermore, a high degree of linkage disequilibrium was observed between the C677T and A1298C polymorphisms, with the D' of 0.887 and $p < 0.01$. The frequencies of haplotypes were 44.7% for MTHFR 677C/1298A, 39.3% for MTHFR 677T/1298A, 15.3% for MTHFR 677C/ 1298C, and 0.7% for MTHFR 677T/1298C respectively. Considering the MTHFR C677T is more likely associated with risk of HCC, it was then selected for further interaction analysis.

We further estimated the possible effect modifications by MTHFR C677T on the effects of major risk factors of HCC. Results are shown in Table 4. No obvious effect modifications

were observed between MTHFR677 and HBsAg or anti-HCV. The adjusted OR for joint effect of the MTHFR any T genotype and moldy food intake was 3.37 (95% CI: 1.75–6.51). Our results suggested a possibility of a more than multiplicative interaction between the MTHFR677 any T genotype and ingestion of moldy food, with a *p* value for interaction of 0.16. The *p* value for joint effect of the MTHFR 677 any T genotype and raw water drinking was 0.03 after adjustment. Subjects with low fruit and vegetable intake and MTHFR 677 any T genotype has an increased risk of HCC by 2.79 times.

Discussion

As it is generally the case with retrospective case–control studies on dietary factors, recall biases may exist, which may lead to an overestimate of any association between the risk factors under study and the disease. On the other hand, the cases may change their dietary pattern after their diagnosis, which might lead to an underestimation of the association under study. For example, cases may report moldy food intake more often than controls if they knew that the moldy food was a major risk factor of liver cancer. However, cases might change their dietary pattern, which might result in reporting reduced intake of moldy food, leading to an underestimation of the association under study. It is very difficult to estimate the direction of those biases on the observation. Besides, the present study has a relatively low participation rate (57%). The major reason is that liver cancer is an invariably fatal disease and a high proportion of those newly diagnosed cases died before our interviewers could reach them. This might be the case of most population-based case–control studies of fatal diseases. The low response of cases may lead to potential selection bias. Cases included in the study may only represent those with relatively less severe conditions in comparison to deceased cases. The results of a study of this kind may represent only the disease at a less advanced stage.

Taixing is one of the areas with the highest HCC prevalence in the world. The incidence of primary HCC is 55.6/100,000 for both genders, 84.6/100,000 for males, and 25.0/100,000 for females in 2000, which far exceeds the average level for all of China. Though most of the risk for HCC can be attributed to environmental risk factors, 20% of HCCs still have an unclear etiology.

MTHFR is a critical enzyme in both DNA synthesis and methylation, thereby affecting DNA stability and gene expression and playing an important role in tumor progression. The frequency of the variant genotype is heterogeneous in different regions of the world. Several studies of Chinese subjects have reported that frequencies of MTHFR 677 C/C, C/T, and T/T were around 31.5~35.0%, 47.8~49.5%, and 15.7~19.0%, respectively, in healthy populations [17, 18, 25, 26], which is similar to the frequencies of 34.5% for C/C, 50.9% for C/T, and 14.6% for T/T in the present study's healthy controls. Among all the studies in Chinese populations, the MTHFR 677 T allele frequency (around 40%) is higher than that observed among Caucasian, African, American, Mexican-American, and Pakistani populations [13, 27, 28]. In the present study, compared with the healthy controls, HCC cases were found to have a higher proportion of C/T and T/T genotypes. MTHFR 677 C/T genotype carriers have a significant higher risk of HCC.

A number of studies explored the possible relationship between MTHFR C677T and cancer risk. However, the results are greatly inconsistent among various as well as within the same cancer sites. Some of these studies reported that MTHFR 677T allele carriers have reduced risks of colorectal [5–9], leukemia [10–12], and lung cancers [29]. In contrast, other studies reported elevated risks of esophageal [17], gastric [18], ovarian [30], cervical [14], bladder [13], colorectal [31, 32], and lung cancers [33] for MTHFR 677T allele carriers. These conflicting results might be explained by the modifying effects of the MTHFR polymorphisms on the balance between DNA methylation and DNA synthesis.

MTHFR catalyzes the reduction of 5,10-methlenetetrahydrolate to 5-methyltetrahydrofolate, the major circulatory form of folate in the body and a carbon donor for conversion of homocysteine to methionine. As a precursor of S-adenosylmethionine (SAM), methionine is the universal methyl donor for DNA methylation [2, 3, 34]. Individuals carrying the variant MTHFR 677TT genotype or 677CT have about 30% or 65% of enzyme activity, respectively, in vitro as compared with the CC wild type [2]. On one hand, low enzyme activity of MTHFR C677T variant genotypes are associated with DNA hypomethylation, which may induce genomic instability and thereby affect the expression of oncogenes or tumor suppressor genes [35–38]. On the other hand, individuals with MTHFR low enzyme activity will have higher plasma homocysteine levels, leading to a great pool of methylene-THF. Enhanced availability of methylene-THF in the DNA-synthesis pathway reduces misincorporation of uracil in DNA. Thus from the standpoint of DNA methylation, the MTHFR 677T allele carrier might increase the risk of developing cancers, whereas from the standpoint of DNA synthesis, MTHFR 677T allele carriers might have a protective effect on cancer risk [13]. The balance may depend on environmental factors, particularly dietary folate intake, which influences not only the available pool of 5, 10-methylene-THF, the substrate for thymidylate synthesis, but also the available pool of S-adenosylmethionine, the universal methyl donor for methyl transferences. Therefore, for subjects with MTHFR 677T allele and low folate intake, both DNA methylation and DNA synthesis might be impaired [39] and may increase cancer risk. However when dietary folate level is adequate, the MTHFR T allele may protect against cancer due to the sufficient methyl donor and beneficial DNA synthesis.

Some studies have reported that folate deficiency is fairly common in many Chinese areas, especially northern China including Beijing [40–43]. In Taixing city, the present study field area, located in the Northern Jiangsu province, the boundary between North and South China, most residents are country people with low consumption of fresh vegetables and fruits due to the relative low economic level. Based on the folate deficiency status, observed increased risk of HCC for T allele carrier could be explained by DNA hypomethylation due to reduced levels of SAM caused by low MTHFR enzyme activity. To date, only one study has reported a significant association between the MTHFR 677C/C genotype and risk of HCC in patients with alcoholic cirrhosis, whereas the HCC patients without alcoholic cirrhosis have a higher proportion of the MTHFR 677T allele compared with healthy subjects and patients with alcoholic cirrhosis without HCC [19]. This conclusion is similar to the results we reported in the current study. Furthermore, some studies conducted in China reported a positive relationship between the MTHFR 677T allele and higher risk of

stomach cancer. The similar trend might suggest that the T allele may increase the risk of upper-GI cancers, especially among Chinese populations with low folate intake.

The further exploration of interactions between MTHFR and environmental risk factor suggests a possible multiplicative interaction with raw water drinking and moldy food intake. The two behavior factors are considered major risk factors of HCC, following HBV infection. Raw water and moldy food are major sources of hepatocarcinogens [44], which may impair liver function. Due to the critical role of the liver in the storage and metabolism of folate, injury to liver function may affect the folate-involved process of DNA methylation and DNA synthesis and interact with MTHFR SNPs, playing an important role in the development of HCC. In addition, somatic mutations caused by these environmental carcinogen exposures may combine with inherited susceptibility (MTHFR any T) and hugely elevate risk of HCC. Still, the relatively small sample size does not allow a well-conducted interaction estimation. The present results can only be used to give the implication for further investigation; we could not make any conclusion based on this preliminary result.

Based on the observation of high degree *of linkage disequilibrium*, it was believed that the association with A1298C may through LD with the C677T and/or other variants.

In summary, this study not only suggests the role of the MTHFR 677 C/T genotype as an independent risk factor but also suggests the polymorphic genotypes as a modifier, modulating the risk of HCC caused by environmental risk factors.

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Table 1

Distribution of demographic characters among cases and controls

Variables	Case (*n*%)	Control (*n*%)	Total (*n*%)	*p* value†
Gender				
Male	159 (77.94)	287 (69.16)	446 (72.05)	0.02
Female	45 (22.06)	128 (30.84)	173 (27.95)	
Total	204	415	619	
Age				
<40	31(15.20)	31 (7.47)	62 (10.02)	<0.01
40–50	54 (26.47)	69 (16.63)	123 (19.87)	
50–60	54 (26.47)	136 (32.77)	190 (30.69)	
60–70	42 (20.59)	116 (27.95)	158 (25.53)	
>70	23 (11.27)	63 (15.18)	86(13.89)	
Total	204	415	619	
Education				
Illiteracy	44 (21.57)	73 (17.59)	117 (18.90)	<0.01‡
Primary	77 (37.75)	142 (34.22)	219 (35.38)	
Middle	70 (34.31)	124 (29.88)	194 (31.34)	
High	13 (6.37)	66 (15.90)	79 (12.76)	
College	0 (0)	10 (2.41)	10 (1.62)	
Total	204	415	619	
Income (per capita)				
<60	63 (30.88)	88 (21.20)	151 (24.39)	0.06
60–100	35 (17.16)	74 (17.83)	109 (17.61)	
100–160	58 (28.43)	135 (32.53)	193 (31.18)	
>160	48 (23.53)	118 (28.43)	166 (26.82)	
Total	204	415	619	

† Based on Chi-square testing

‡ Fisher's exact testing

Table 2

Potential risk factors among cases and controls

Variables	Case *n*%	Control *n*%	Adjusted OR & 95% CI‡
HBV infection			
HBsAg−	72(35.29)	312(75.36)	1
HBsAg+	132(64.71)	102(24.64)	5.14(3.50~7.55)
	204	414	
HCV infection			
Anti-HCV−	183(91.04)	403(97.11)	1
Anti-HCV+	18(8.96)	12(2.89)	3.39(1.43~8.03)
	201	415	
Moldy food intake			
No	143(74.87)	339(83.70)	1
Yes	48(25.13)	66(16.30)	2.25(1.39~3.64)
	191	405	
Raw water drinking			
Never	95(51.4)	248(69.08)	1
Few/ever	27(14.6)	44(12.26)	1.47(0.80~2.71)
Sometimes	52(28.11)	62(17.27)	1.89(1.15~3.09)
Often	11(5.95)	5(1.39)	4.60(1.30~16.30)
	185	359	$p^{\dagger} < 0.01$
Tobacco smoking			
Never	85 (44.27)	217 (52.42)	1
Ever	107 (55.73)	197 (47.58)	1.12(0.70~1.81)
	192	414	
Cigarettes per day			
Never	85(44.27)	217(52.42)	1
<20	66(34.38)	128(30.92)	1.06(0.63~1.78)
20~30	34(17.71)	61(14.73)	1.18(0.64~2.18)
≥30	7(3.65)	8(1.93)	1.73(0.50~5.99)
	192	414	$p^{\dagger} = 0.41$
Alcohol drinking			
Never/few	116(60.42)	279(67.72)	1
Often	51(26.56)	75(18.20)	1.43(0.86~2.36)
Everyday	25(13.02)	58(14.08)	1.12(0.62~2.05)
	201	412	$p^{\dagger} = 0.45$
Family history of liver cancer			
No	150(73.89)	375(90.58)	1
Yes	53(26.11)	39(9.42)	2.99(1.78~5.01)
	183	414	
Quantity of green tea drinking per month			

Variables	Case *n*%	Control *n*%	Adjusted OR & 95% CI‡
No	111(60.33)	216(54.41)	1
<125 g	23(12.85)	42(11.11)	1.23(0.64~2.36)
125~250 g	24(13.41)	50(13.23)	0.88(0.46~1.69)
≥250 g	21(11.73)	70(18.52)	0.59(0.31~1.12)
	179	378	$p^{\dagger} = 0.13$
Fruit and vegetable intake			
Low	141(69.1)	228(54.9)	
High	63(30.9)	187(45.1)	0.58(0.38~0.89)
	204	415	

† *p* value for trend

‡ Adjusted on age (continuous variable), gender (male or female), education, HbsAg

Table 3

Polymorphisms of MTHFR and the risk of primary liver cancer

Polymorphic sites		Case *n*(%)	Control *n*(%)	Adjusted OR & 95% CI‡
MTHFR677				
C/C		50 (25.77)	135 (34.53)	1
C/T		114 (58.76)	199 (50.90)	1.66 (1.06–2.61)
T/T		30 (15.46)	57 (14.58)	1.21 (0.65–2.28)
				p_{trend} = 0.25
C/C		50 (25.77)	135 (34.53)	1
C/T or T/T(Any T)		144 (74.23)	256 (65.47)	1.55 (1.01–2.40)
		194	391	
MTHFR1298				
A/A		135(69.59)	275(69.79)	1
A/C		55(28.35)	112(28.43)	1.00(0.65~1.55)
C/C		4(2.06)	7(1.78)	1.38(0.33~5.75)
				p = 0.84
A/A		135(69.59)	275(69.79)	1
A/C or C/C (Any C)		59(30.41)	119(30.21)	1.02(0.67~1.57)
		194	394	
MTHFR677	MTHFR1298			
C/C	A/A	22(11.34)	71(18.16)	1
C/C	A/C or C/C	29(14.95)	64(16.37)	1.72(0.83~3.59)
C/T or T/T	A/A	112(57.73)	202(51.66)	2.03(1.12~3.68)
C/T or T/T	A/C or C/C	31(15.98)	54(13.81)	1.94(0.93~4.06)

‡Adjusted on age (continuous variable), gender (male or female), education (continuous), HBsAg

*The observed genotype frequency among the control subjects was in agreement with the Hardy-Weinberg equilibrium (Σx^2 =1.3353, $p > 0.05$ for the MTHFR C677T, Σx^2 =1.3515, $p > 0.05$ for the MTHFR A1298C)

Table 4

Gene-environmental interactions between MTHFR and other risk factors on the risk of primary liver cancer

MTHFR	Environmental factors	Case *n*	Control *n*	Adjusted OR (95% CI)‡
C677T	HbsAg			
C/C	−	16	102	1
C/C	+	34	32	6.05(2.88~12.71)
Any T	−	43	190	1.66(0.89~3.12)
Any T	+	95	66	8.80(4.66~16.63)
p value for interaction			0.76	
C677T	Anti-HCV			
C/C	−	49	134	1
C/C	+	1	1	4.16(0.03~602.82)
Any T	−	127	245	1.43(0.92~2.23)
Any T	+	16	11	4.11(1.60~10.55)
p value for interaction			0.89	
C677T	Moldy Food Intake			
C/C	No	41	109	1
C/C	Yes	8	23	1.23(0.45~3.33)
Any T	No	94	209	1.21(0.74~1.98)
Any T	Yes	38	40	3.37(1.75~6.51)
p value for interaction			0.16	
C677T	Raw water drinking			
C/C	No	35	89	1
C/C	Yes	9	26	0.72(0.28~1.85)
Any T	No	83	182	1.14(0.67~1.94)
Any T	Yes	50	41	2.78(1.46~5.31)
p value for interaction			0.03	
C677T	Cigarette smoking			
C/C	No	23	66	1
C/C	Yes	26	69	0.83(0.37~1.87)
Any T	No	58	136	1.17(0.61~2.23)
Any T	Yes	75	119	1.50(0.73~3.07)
p value R For interaction			0.34	
C677T	Alcohol drinking			
C/C	Never/Few	29	91	1
C/C	Often/Everyday	20	44	1.84(0.84~4.06)
Any T	Never/Few	82	171	1.80(1.03~3.15)
Any T	Often/Everyday	51	82	1.94(1.01~3.72)
p value for interaction			0.25	
C677T	Green tea drinking			
C/C	Yes	21	56	1
C/C	No	27	72	0.94(0.43~2.06)

MTHFR	Environmental factors	Case *n*	Control *n*	Adjusted OR (95% CI)‡
Any T	Yes	47	113	1.01(0.52~1.97)
Any T	No	79	133	1.59(0.81~3.12)
p value for interaction			0.26	
C677T	Fruit and vegetable			
C/C	High	13	55	1
C/C	Low	37	80	1.67(0.74~3.79)
Any T	High	50	120	1.59(0.73~3.48)
Any T	Low	94	136	2.79(1.31~5.97)
p value for interaction			0.92	

‡Adjusted on age (continuous variable), gender (male or female), education (continuous), HBsAg