Research Article

Association between methylenetetrahydrofolate reductase tagging polymorphisms and susceptibility of hepatocellular carcinoma: a case–control study

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Polymorphisms in one-carbon metabolism genes may influence the susceptibility to hepatocellular carcinoma (HCC). In the present study, we studied methylenetetrahydrofolate reductase (MTHFR) tagging polymorphisms in 584 HCC cases and 923 controls. Polymerase chain reaction was harnessed to detect MTHFR genotype. Overall, our results showed that genotype distribution of MTHFR rs4846048 and rs4845882 polymorphisms was not different between HCC patients and controls. MTHFR rs9651118 and rs1801133 loci were protective factors for HCC (rs9651118: CT vs. TT: adjusted odds ratio (OR) = 0.67, 95% confidence interval (CI): 0.49–0.90, P = 0.008 and TC/CC vs. TT: adjusted OR = 0.70, 95% CI: 0.53–0.93, P = 0.015; rs1801133: GA vs. GG: adjusted OR = 0.72, 95% CI: 0.54–0.97, P = 0.031, AA/GA vs. GG: adjusted OR = 0.76, 95% CI: 0.57–0.99, P = 0.045). However, MTHFR rs3753584 locus was a candidate for susceptibility to HCC (CT vs. TT: adjusted OR = 1.67, 95% CI: 1.20–2.32, P = 0.003 and TC/CC vs. TT: adjusted OR = 1.59, 95% CI: 1.15–2.07, P = 0.005). Results of haplotype analysis suggested that MTHFR G<sub>rs1801133</sub>T<sub>rs3753584</sub>G<sub>rs4845882</sub>A<sub>rs4846048</sub>T<sub>rs9651118</sub> was associated with the risk of HCC (OR = 1.55, 95% CI: 1.16–2.07, P = 0.003). The power of our study also confirmed these associations (the value of power > 0.80). In summary, our findings suggested that MTHFR rs3753584, rs9651118 and rs1801133 polymorphisms may affect the risk of HCC in Chinese Han population. In future, our findings should be further validated in additional case–control studies.

Introduction

In 2015, liver cancer (LC) ranked the third most frequent type of malignancy in males and the sixth most frequent type in females, approximately 343700 and 122300 cases occurring in China, respectively [1]. The total LC-related deaths are the third most frequent type of malignancy [1,2]; however, the etiology of LC remains unclear. Susceptibility factors [e.g. hepatitis B virus (HBV), age, obesity, type 2 diabetes, consumption of food contaminated with aflatoxin, nonalcoholic fatty liver disease, heavy drinking related cirrhosis, and tobacco use] may be implicated in the etiology of LC [3–7]. Accumulating evidences suggested that besides these mentioned people's lifestyle and environmental factors, some genetic predispositions might also contribute to development of LC.

Folic acid is important for DNA synthesis and methylation, mitosis and controlling related gene expression. Recently, epidemiological investigations showed that sufficient fruits and vegetables intake may be a protective factor for carcinogenesis [8–10]. It is thought that these potential protective roles of diet attribute to the high level intake of folic acid. Methylenetetrahydrofolate reductase (MTHFR) plays an important role in catalyzing the transition of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate...
(5-MTHF), which is a main plasm form of folate. And 5-MTHF is implicated in a conversion procedure of homocysteine (Hcy) into methionine (Met) and the methylation of DNA. Considering the vital role of MTHFR, variants in MTHFR gene may influence the development of cancer.

In humans, MTHFR protein is coded by the MTHFR gene which is located on chromosome 1p36.3. MTHFR single nucleotide polymorphisms (SNPs) may be a potential biomarker of cancer. In addition, Jiao et al. [11] reported that hepatocellular carcinoma (HCC) patients with HBV-infection carried MTHFR rs1801133 AA genotype and A allele may have a better prognosis than those who carried MTHFR rs1801133 GG genotype and G allele. Recently, some studies investigated a potential association of MTHFR loci with HCC susceptibility [11–15]; however, due to the limited sample sizes, the observations might be conflicting. Therefore, in the present study, we included 1507 subjects to perform a case–control study to extensively explore the relationship between MTHFR tagging SNPs and the risk of HCC.

Materials and methods

Study population

Our study recruited 584 consecutive HCC cases from two Clinical Medical College of Fujian Medical University (Fuzhou City, China) during 2002–2016. Two doctors confirmed HCC diagnosis by pathology. The criterion of Barcelona Clinic Liver Cancer (BCLC) was used to determine the stage of HCC [16,17]. In addition, 923 Chinese people without any cancer history were included as controls. We matched HCC patients and controls by region (Eastern China), age and sex. Every subject was notified purpose of the study. All participants provided written informed consent. A questionnaire regarding age, sex, smoking and alcohol status was used to collect the corresponding information. The protocol of this investigation was approved by Ethics Committee of Fujian Medical University. In the present study, the principles of Declaration of Helsinki was conformed. Chronic HBV infection were determined by using hepatitis B surface antigen enzyme-linked immunosorbent assay Kit (InTec, Xiamen, China).

SNPs selection and genotyping

MTHFR tagging SNPs were selected through Haploview software, which are described in our previous case–control studies [18,19].

EDTA-anticoagulated blood was donated and collected. According to the standard experimental protocol, genomic DNA was obtained by using a DNA Kit (Promega, Madison, U.S.A.). A SNPscan Kit (Genesky Biotechnologies Inc., Shanghai, China) was used to determine the variants of MTHFR SNPs as described in our case–control studies [20]. At this stage, 60 DNA samples were selected and re-analyzed for quality control. Finally, the genotype frequencies were not changed.

Statistical analysis

In the present study, χ² test or Fisher’s exact test was harnessed to analyze the differences in sex, chronic HBV infection, age, smoking, drinking and the genotype frequencies of HCC patients compared with controls. In addition, age was expressed as means ± standard deviation (SD). The difference in age between two groups was determined by Student’s t test. With an internet Hardy–Weinberg equilibrium (HWE) test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl), genotype frequencies among controls was used to assess the HWE status [21,22,23,24]. The correlation between MTHFR SNPs and HCC risk was evaluated by odds ratios (ORs) and 95% confidence intervals (CIs). Logistic regression analysis was used to determine the associations between MTHFR polymorphisms and the risk of HCC by adjusting sex, chronic HBV infection, age, smoking and drinking. A two-tailed P<0.05 was considered as significant. We used SHE-SIS online program (http://analysis.bio-x.cn/myAnalysis.php) to construct MTHFR haplotypes [25]. SAS 9.4 version statistical software (SAS Institute, Cary, NC) was used to analyze data. The power of the present study was determined by a power calculating software (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize) (α = 0.05) [26].

Results

Characteristics

As described in Table 1, 1507 participants (584 patients and 923 controls) were included in the present study. Male:female ratio of the HCC patients was ~9:1 (89.90% and 10.10%), controls were recruited in the similar proportion (90.47% males and 9.53% females) to match the distribution of sex and average age (HCC cases = 53.17, SD ± 11.76 years; controls = 53.72, SD ± 9.97 years; P=0.327). There were 210 (35.96%) smokers among HCC group.
Table 1 Distribution of selected demographic variables and risk factors in HCC cases and controls

| Variable                  | Cases (n=584) | Controls (n=923) | P
|---------------------------|--------------|------------------|---
| Age (years)               | 53.17 (±11.76) | 53.72 (±9.97) | 0.327
| Age (years)               |              |                  | 0.358
| <53                       | 264 (45.21)  | 395 (42.80)     |              
| ≥53                       | 320 (54.79)  | 528 (57.20)     |              
| Sex                       |              |                  | 0.717
| Male                      | 525 (89.90)  | 835 (90.47)     |              
| Female                    | 59 (10.10)   | 88 (9.53)       |              
| Smoking status            |              |                  |              
| Never                     | 374 (64.04)  | 596 (64.57)     |              
| Ever                      | 210 (35.96)  | 327 (35.43)     |              
| Alcohol use               |              |                  |              
| Never                     | 414 (70.89)  | 775 (83.97)     |              
| Ever                      | 170 (29.11)  | 148 (16.03)     |              
| Chronic HBV infection     |              |                  |              
| Yes                       | 412 (70.55)  | 85 (9.21)       |              
| No                        | 172 (29.45)  | 838 (90.79)     | 0.834

Bold values are statistically significant (P<0.05).

1 Two-sided χ² test and Student’s t test.

Table 2 Primary information for MTHFR polymorphisms

| Genotyped SNPs | rs3753584 T>C | rs4846048 A>G | rs4845882 G>A | rs1801133 G>A | rs9651118 T>C |
|----------------|--------------|--------------|--------------|--------------|--------------|
| Chromosome     | 1            | 1            | 1            | 1            | 1            |
| Location       | 11864586     | 11846252     | 11843167     | 11856378     | 11862214     |
| Regulome DB     | NearGene-5   | Intron       | Intron       | Missense     | Intron       |
| Transcription   | Y            | -            | -            | -            | Y            |
| MIRNA           | -            | Y            | -            | -            | -            |
| MAF for Chinese | 0.093        | 0.105        | 0.198        | 0.439        | 0.382        |
| MAF in controls | 0.111        | 0.095        | 0.216        | 0.354        | 0.378        |
| P-value for     | 0.814        | 0.029        | 0.437        | 0.074        | 0.021        |
| Genotyping      | SNPscan      | SNPscan      | SNPscan      | SNPscan      | SNPscan      |
| % Genotyping    | 99.27%       | 99.27%       | 99.27%       | 99.27%       | 99.27%       |

1 MAF, minor allele frequency.

and 327 (35.43%) among non-cancer controls, while non-smokers were 374 (64.04%) in HCC cases and 596 (64.57%) in the controls. Drinking and chronic HBV infection ratio in HCC patients was higher than that of controls (29.11 vs. 16.03% and 70.55 vs. 9.21%, respectively). The BCLC stage of HCC was summarized in Table 1. The data of SNPs in MTHFR gene were listed in Table 2.

Relationship of MTHFR polymorphisms with HCC patients

Table 3 summarizes the MTHFR genotype frequencies in HCC patients and control groups. Overall, we found that MTHFR rs4846048 and rs4845882 genotype distribution were not statistically significant between two groups.
Table 3 Logistic regression analyses of associations between MTHFR rs3753584 T>C, rs4845882 G>A, rs1801133 G>A, rs4846048 A>G and rs9651118 T>C polymorphisms and the risk of HCC

| Genotype          | Cases (n=584) | Controls (n=923) | Crude OR (95% CI) | P     | Adjusted OR1 (95% CI) | P       |
|-------------------|---------------|------------------|-------------------|-------|-----------------------|---------|
|                   | n             | %                | n                 | %     |                       |         |
| MTHFR rs1801133   |               |                  |                   |       |                       |         |
| G>A               |               |                  |                   |       |                       |         |
| GG                | 299           | 52.00            | 372               | 40.39 | 1.00                  | 1.00    |
| GA                | 227           | 39.48            | 446               | 48.43 | 0.63 (0.51–0.79) <0.001| 0.72 (0.54–0.97) 0.031 |
| AA                | 49            | 8.52             | 103               | 11.18 | 0.59 (0.41–0.86) 0.006| 0.89 (0.66–1.42) 0.625 |
| GA+AA             | 276           | 48.00            | 549               | 59.48 | 0.63 (0.51–0.77) <0.001| 0.76 (0.57–0.99) 0.045 |
| GG+GA             | 526           | 91.48            | 818               | 88.82 | 1.00                  | 1.00    |
| AA                | 49            | 8.52             | 103               | 11.18 | 0.74 (0.52–1.06) 0.098| 1.05 (0.67–1.64) 0.842 |
| A allele          | 325           | 28.26            | 652               | 35.40 |                       |         |
| MTHFR rs3753584   |               |                  |                   |       |                       |         |
| T>C               |               |                  |                   |       |                       |         |
| TT                | 431           | 74.96            | 729               | 79.15 | 1.00                  | 1.00    |
| CT                | 139           | 24.17            | 180               | 19.54 | 1.31 (1.02–1.68) 0.037| 1.67 (1.20–2.32) 0.003 |
| CC                | 5             | 0.87             | 12                | 1.30  | 0.71 (0.25–2.01) 0.514| 0.64 (0.16–2.57) 0.530 |
| CT+CC             | 144           | 25.04            | 192               | 20.85 | 1.27 (0.99–1.62) 0.059| 1.59 (1.15–2.20) 0.005 |
| TT+CT             | 570           | 99.13            | 909               | 99.70 | 1.00                  | 1.00    |
| CC                | 5             | 0.87             | 12                | 1.30  | 0.67 (0.23–1.90) 0.445| 0.58 (0.15–2.29) 0.434 |
| C allele          | 149           | 12.96            | 204               | 11.07 |                       |         |
| MTHFR rs4845882   |               |                  |                   |       |                       |         |
| G>A               |               |                  |                   |       |                       |         |
| GG                | 329           | 57.22            | 562               | 61.02 | 1.00                  | 1.00    |
| GA                | 222           | 38.61            | 320               | 34.74 | 1.19 (0.96–1.48) 0.128| 1.26 (0.94–1.67) 0.121 |
| AA                | 24            | 4.17             | 39                | 4.23  | 1.05 (0.62–1.78) 0.853| 1.18 (0.59–2.36) 0.650 |
| GA+AA             | 246           | 42.78            | 359               | 38.98 | 1.17 (0.95–1.45) 0.145| 1.25 (0.94–1.65) 0.120 |
| GG+GA             | 551           | 95.83            | 882               | 95.77 | 1.00                  | 1.00    |
| AA                | 24            | 4.17             | 39                | 4.23  | 0.99 (0.59–1.66) 0.955| 1.08 (0.54–2.15) 0.831 |
| A allele          | 270           | 23.48            | 398               | 21.61 |                       |         |
| MTHFR rs4846048   |               |                  |                   |       |                       |         |
| A>G               |               |                  |                   |       |                       |         |
| AA                | 465           | 80.87            | 760               | 82.52 | 1.00                  | 1.00    |
| AG                | 107           | 18.61            | 147               | 15.96 | 1.19 (0.90–1.57) 0.215| 1.17 (0.82–1.68) 0.395 |
| GG                | 3             | 0.52             | 14                | 1.52  | 0.35 (0.10–1.23) 0.101| 0.26 (0.06–1.23) 0.090 |
| AG+GG             | 110           | 19.13            | 161               | 17.48 | 1.12 (0.85–1.46) 0.421| 1.08 (0.76–1.54) 0.668 |
| AA+AG             | 572           | 94.83            | 907               | 95.49 | 1.00                  | 1.00    |
| GG                | 3             | 0.52             | 14                | 1.52  | 0.34 (0.10–1.19) 0.091| 0.25 (0.05–1.20) 0.083 |
| G allele          | 113           | 9.82             | 175               | 9.50  |                       |         |
| MTHFR rs9651118   |               |                  |                   |       |                       |         |
| T>C               |               |                  |                   |       |                       |         |
| TT                | 216           | 37.57            | 340               | 36.92 | 1.00                  | 1.00    |
| TC                | 267           | 46.43            | 466               | 50.60 | 0.90 (0.72–1.13) 0.373| 0.67 (0.49–0.90) 0.008 |
| CC                | 92            | 16.00            | 115               | 12.49 | 1.26 (0.91–1.74) 0.162| 0.85 (0.55–1.31) 0.458 |
| TC+CC             | 359           | 62.43            | 581               | 63.08 | 0.97 (0.78–1.21) 0.800| 0.70 (0.53–0.93) 0.015 |
| TT+TC             | 483           | 84.00            | 806               | 87.51 | 1.00                  | 1.00    |
| CC                | 92            | 16.00            | 115               | 12.49 | 1.34 (0.99–1.80) 0.056| 1.07 (0.72–1.59) 0.758 |
| C allele          | 451           | 39.22            | 696               | 37.79 |                       |         |

1 Adjusted for age, sex, smoking, status of chronic HBV infection and drinking. Bold values are statistically significant (P<0.05).

Compared with rs1801133 GG genotype frequency, we found that MTHFR rs1801133 GA genotype significantly decreased the risk of HCC (P<0.001). When rs1801133 GG frequency was used as reference, there was a difference in MTHFR rs1801133 AA and AA/GA genotype frequency between two groups (P=0.006 and P<0.001, respectively). Adjustment for sex, chronic HBV infection, age, smoking and drinking, the significant decreased risk also re-appeared in two genetic models (GA vs. GG: adjusted P=0.031, AA/GA vs. GG: adjusted P=0.045, respectively).
We also found MTHFR rs3753584 had an increased susceptibility to HCC (CT vs. TT: \( P=0.037 \)). Adjustments for sex, chronic HBV infection, age, smoking and drinking, the findings were more significant (CT vs. TT: adjusted \( P=0.003 \) and TC/CC vs. TT: adjusted \( P=0.005 \)).

In crude comparison, we did not find any relationship between MTHFR rs9651118 and HCC susceptibility. However, when adjusted for sex, chronic HBV infection, age, smoking and drinking, a statistically decreased risk of HCC was identified in two genetic model (CT vs. TT: adjusted \( P=0.008 \) and TC/CC vs. TT: adjusted \( P=0.015 \)).

**Association of MTHFR polymorphisms with HCC in a stratification analysis**

Table 4 showed the MTHFR genotype frequencies in the subgroup analyses by the status of chronic HBV infection. We found that MTHFR rs1801133 and rs9651118 polymorphisms were associated with the decreased risk of HCC in no chronic HBV infection subgroup (rs1801133: GA vs. GG: adjusted \( P=0.001 \) and GA/AA vs. GG: adjusted \( P=0.001 \); and rs9651118: CT vs. TT: adjusted \( P=0.021 \) and TC/CC vs. TT: adjusted \( P=0.037 \)). However, we identified that MTHFR rs1801133 polymorphism was associated with HCC risk in chronic HBV infection subgroup (rs1801133: AA vs. GG: adjusted \( P=0.035 \) and GA/AA vs. GG: adjusted \( P=0.035 \)). Additionally, the association of MTHFR rs3753584 and rs4845882 polymorphism with the risk of HCC was also found (rs3753584: CT vs. TT: adjusted \( P=0.003 \) and TC/CC vs. TT: adjusted \( P=0.005 \); rs4845882: GA vs. GG: adjusted \( P=0.022 \) and GA/AA vs. GG: adjusted \( P=0.021 \)).

**MTHFR haplotypes**

We constructed six haplotypes of MTHFR gene (Table 5). Haplotype analysis of this gene suggested that MTHFR \( A_{rs1801133}T_{rs3753584}G_{rs4845882}A_{rs4846048}T_{rs9651118} \) haplotype was a protective factor for HCC (\( P=0.008 \)). However, MTHFR \( G_{rs1801133}T_{rs3753584}G_{rs4845882}A_{rs4846048}T_{rs9651118} \) was associated the risk of HCC (\( P=0.003 \)).

**The power of the present study (\( \alpha = 0.05 \))**

The present study’s power was determined (\( \alpha = 0.05 \)). For MTHFR rs1801133 polymorphism, the power value was 0.835 in the GA vs. GG genetic model and 0.726 in GA/AA vs. GG genetic model. For MTHFR rs3753584 locus, the power of the present study was 0.984 in the CT vs. TT genetic model and 0.965 in CT/CC vs. TT genetic model. When we focused on the MTHFR rs9651118 polymorphism, the power value was 0.935 in the CT vs. TT genetic model and 0.910 in CT/CC vs. TT genetic model. In addition, the power of MTHFR \( A_{rs1801133}T_{rs3753584}G_{rs4845882}A_{rs4846048}T_{rs9651118} \) and MTHFR \( G_{rs1801133}T_{rs3753584}G_{rs4845882}A_{rs4846048}T_{rs9651118} \) haplotypes were 0.771 and 0.844, respectively.

In the subgroup without chronic HBV infection, we found that the power value was 0.946 in GA vs. GG genetic model and 0.944 in GA/AA vs. GG genetic model for MTHFR rs1801133 polymorphism and 0.852 in the CT vs. TT genetic model and 0.803 in CT/CC vs. TT genetic model for MTHFR rs3753584 locus. The power value of other subgroups was less than 0.8 (data were not shown).

**Discussion**

The HCC susceptibility to individuals may be affected by certain environmental risk factors [27,28]. The high HCC morbidity in certain regions of sub-Saharan Africa and Asia largely attributes to the prevalence of chronic HBV infection. However, individual’s hereditary factor also could influence the risk of HCC [28,29]. MTHFR and 5-MTHF may be implicated in DNA methylation, synthesis and repair. Thus, variants in MTHFR that could influence the risk of cancer. Several case–control studies were designed to identify the association of MTHFR variants with HCC risk. However, the included participants in these studies were relatively small. In addition, the observations of pooled analyses were conflicting [30-33]. Here, we conducted a study with related large sample sizes to assess a potential correlation between MTHFR SNPs and susceptibility of HCC. Our results suggested the associations of MTHFR rs3753584, rs9651118 and rs1801133 polymorphisms with HCC development. In addition, haplotype analysis of MTHFR gene suggested that MTHFR \( G_{rs1801133}T_{rs3753584}G_{rs4845882}A_{rs4846048}T_{rs9651118} \) increased the susceptibility of HCC. The power value of the present study also conferred these associations (power value > 0.80).

Rs1801133 polymorphism is the most extensively studied SNP in MTHFR gene. This SNP is a missense variant (Ala→Val at 226 position). MTHFR is vital enzyme in the process of remethylation, and catalyzes Hcy to Met. Rs1801133 locus codes the NH₂-terminal catalytic domain of MTHFR. MTHFR rs1801133 A allele decreases the activity of protein enzyme [34]. A few case–control studies identified that rs1801133 increased the susceptibility of HCC [15,35,36], Another study identified that this SNP did not confer risk to HCC [37]. However, Jiao et al. [11] reported that AA genotype and A allele of MTHFR rs1801133 may confer a protective effect on HCC in HBV-infected...
| Genotype | Chronic HBV infection (Yes) | Chronic HBV infection (No) |
|----------|-----------------------------|---------------------------|
|          | **Case** | **Control** | **Adjusted OR** | **P** | **Case** | **Control** | **Adjusted OR** | **P** |
| MTHFR rs1801133 |        |            |               |     |        |            |               |     |
| G>A      |        |            |               |     |        |            |               |     |
| GG       | 210    | 51.98      | 60.00         | 1.00 | 89     | 52.05      | 321            | 38.40 | 1.00 |
| GA       | 163    | 40.35      | 37.65         | 1.53 | (0.91–2.79) | 0.111 | 64             | 37.43 | 414            | 49.52 | 0.53 (0.37–0.76) | 0.001 |
| AA       | 31     | 7.67       | 2.35          | 5.06 | (1.12–22.88) | 0.035 | 18             | 10.53 | 101            | 12.08 | 0.63 (0.36–1.10) | 0.104 |
| AA+GA    | 194    | 48.02      | 40.00         | 1.73 | (1.04–2.87) | 0.035 | 82             | 47.95 | 515            | 61.60 | 0.55 (0.39–0.77) | 0.001 |
| GG+GA    | 373    | 92.33      | 97.65         | 1.00 |        |            |               |     | 153             | 89.47 | 735            | 87.92 | 1.00 |
| AA       | 31     | 7.67       | 2.35          | 4.20 | (0.95–18.67) | 0.059 | 18             | 10.53 | 101            | 12.08 | 0.86 (0.50–1.47) | 0.581 |
| A allele | 225    | 27.85      | 21.18         |        |            |               |               |     | 100             | 29.24 | 616            | 36.84 | 1.00 |
| MTHFR rs3753584 |        |            |               |     |        |            |               |     |
| T>C      |        |            |               |     |        |            |               |     |
| TT       | 312    | 77.23      | 81.18         | 1.00 |        |            |               |     | 119             | 69.59 | 660            | 78.95 | 1.00 |
| CT       | 88     | 21.78      | 17.65         | 1.19 | (0.62–2.28) | 0.600 | 51             | 29.82 | 165            | 19.74 | 1.78 (1.22–2.60) | 0.003 |
| CC       | 4      | 0.99       | 1.18          | 0.69 | (0.07–6.69) | 0.748 | 1              | 0.58  | 11             | 1.32  | 0.50 (0.06–3.95) | 0.912 |
| CT+CC    | 92     | 22.77      | 18.82         | 1.15 | (0.62–1.21) | 0.655 | 52             | 30.41 | 176            | 21.05 | 1.70 (1.17–2.46) | 0.005 |
| TT+CT    | 400    | 99.01      | 98.82         | 1.00 |        |            |               |     | 170             | 99.42 | 825            | 98.68 | 1.00 |
| CC       | 4      | 0.99       | 1.18          | 0.67 | (0.07–6.43) | 0.725 | 1              | 0.58  | 11             | 1.32  | 0.44 (0.06–3.42) | 0.428 |
| C allele | 96     | 11.88      | 10.00         |        |            |               |               |     | 53              | 15.50 | 187            | 11.18 | 1.00 |
| MTHFR rs4850882 |        |            |               |     |        |            |               |     |
| T>C      |        |            |               |     |        |            |               |     |
| TT       | 312    | 77.23      | 81.18         | 1.00 |        |            |               |     | 119             | 69.59 | 660            | 78.95 | 1.00 |
| CT       | 88     | 21.78      | 17.65         | 1.19 | (0.62–2.28) | 0.600 | 51             | 29.82 | 165            | 19.74 | 1.78 (1.22–2.60) | 0.003 |
| CC       | 4      | 0.99       | 1.18          | 0.69 | (0.07–6.69) | 0.748 | 1              | 0.58  | 11             | 1.32  | 0.50 (0.06–3.95) | 0.912 |
| CT+CC    | 92     | 22.77      | 18.82         | 1.15 | (0.62–1.21) | 0.655 | 52             | 30.41 | 176            | 21.05 | 1.70 (1.17–2.46) | 0.005 |
| TT+CT    | 400    | 99.01      | 98.82         | 1.00 |        |            |               |     | 170             | 99.42 | 825            | 98.68 | 1.00 |
| CC       | 4      | 0.99       | 1.18          | 0.67 | (0.07–6.43) | 0.725 | 1              | 0.58  | 11             | 1.32  | 0.44 (0.06–3.42) | 0.428 |
| C allele | 96     | 11.88      | 10.00         |        |            |               |               |     | 53              | 15.50 | 187            | 11.18 | 1.00 |
| MTHFR rs4850484 |        |            |               |     |        |            |               |     |
| T>C      |        |            |               |     |        |            |               |     |
| TT       | 312    | 77.23      | 81.18         | 1.00 |        |            |               |     | 119             | 69.59 | 660            | 78.95 | 1.00 |
| CT       | 88     | 21.78      | 17.65         | 1.19 | (0.62–2.28) | 0.600 | 51             | 29.82 | 165            | 19.74 | 1.78 (1.22–2.60) | 0.003 |
| CC       | 4      | 0.99       | 1.18          | 0.69 | (0.07–6.69) | 0.748 | 1              | 0.58  | 11             | 1.32  | 0.50 (0.06–3.95) | 0.912 |
| CT+CC    | 92     | 22.77      | 18.82         | 1.15 | (0.62–1.21) | 0.655 | 52             | 30.41 | 176            | 21.05 | 1.70 (1.17–2.46) | 0.005 |
| TT+CT    | 400    | 99.01      | 98.82         | 1.00 |        |            |               |     | 170             | 99.42 | 825            | 98.68 | 1.00 |
| CC       | 4      | 0.99       | 1.18          | 0.67 | (0.07–6.43) | 0.725 | 1              | 0.58  | 11             | 1.32  | 0.44 (0.06–3.42) | 0.428 |
| C allele | 96     | 11.88      | 10.00         |        |            |               |               |     | 53              | 15.50 | 187            | 11.18 | 1.00 |

1 Adjusted for age, sex, smoking, status of chronic HBV infection and drinking. Bold values are statistically significant (P<0.05).

individuals. Some pooled analysis investigated a potential correlation of MTHFR rs1801133 with HCC risk. Several meta-analyses reported that MTHFR rs1801133 A allele might increase the risk of HCC [31–33,38]. However, in another meta-analysis, Qin et al. [30] suggested that there was no significant association between MTHFR rs1801133 locus and HCC risk. The observations were controversial. Thus, we conducted a related large sample size study to...
investigate the correlation of rs1801133 locus with HCC risk. We concluded that rs1801133 A allele was a protective factor for HCC. Recently, some pooled-analyses demonstrated that this locus is protective for the development of colorectal cancer in Asians [39,40]. An Ala→Val substitute at 226 position in MTHFR may increase the 5,10-methylenetetrahydrofolate for DNA synthesis [41,42], which may be protective for cancer development. In the future, more studies should be conducted to identify whether G→A variant in MTHFR rs1801133 locus is a protective factor for HCC development.

Table 5 MTHFR haplotype frequencies (%) and risk of HCC

| Haplotypes                                      | HCC (n=1151) | Controls (n=1841) | Crude OR (95% CI) | P   |
|------------------------------------------------|--------------|-------------------|-------------------|-----|
|                                               | n            | %                 | n                 | %   |     |
| rs1801133rs3753584rs4845882rs4846048rs9651118 | 438          | 38.05             | 685               | 37.21 | 1.00 |
| rs1801133rs3753584rs4845882rs4846048rs9651118 | 317          | 27.54             | 633               | 34.38 | 0.78 (0.65–0.94) | 0.008 |
| rs1801133rs3753584rs4845882rs4846048rs9651118 | 140          | 12.63             | 194               | 10.54 | 1.13 (0.88–1.45) | 0.339 |
| rs1801133rs3753584rs4845882rs4846048rs9651118 | 111          | 9.64              | 112               | 6.08  | 1.55 (1.16–2.07) | 0.003 |
| rs1801133rs3753584rs4845882rs4846048rs9651118 | 109          | 9.47              | 170               | 9.23  | 1.00 (0.77–1.31) | 0.984 |
| rs1801133rs3753584rs4845882rs4846048rs9651118 | 13           | 1.13              | 22                | 1.20  | 0.92 (0.46–1.85) | 0.824 |
| Others                                         | 23           | 2.00              | 25                | 1.36  | 1.44 (0.81–2.57) | 0.216 |

Bold values are statistically significant (P<0.05).

To our knowledge, we first clarified the impact of MTHFR rs3753584 T>C polymorphism with hepatocarcinogenesis. MTHFR rs3753584 is located in nearGene-5, which may regulate the stability, transcription and translation of RNA. Liu et al. [43] suggested that MTHFR rs3753584 locus affected the development of lung cancer. Another study found that MTHFR rs3753584 variants increased the susceptibility of colon cancer [44]. Here, we identified that MTHFR rs3753584 may confer a risk to HCC. Our observation was similar to those findings mentioned above.

Lu et al. [45] conducted a study to detect the correlation of MTHFR rs9651118 with susceptibility to breast cancer (BC), and the results suggested that rs9651118 CC genotype decreased the risk of BC. Additionally, in Caucasians, Swartz et al. [46] reported that this variant might be a factor that decreased the susceptibility of lung cancer. In Asians, Ding et al. [47] reported that MTHFR rs9651118 T>C polymorphism has a tendency to decrease risk of esophagogastric junction adenocarcinoma. In the present study, we first explored the association between MTHFR rs9651118 T>C polymorphism and risk of HCC. Our findings clarified that MTHFR rs9651118 C allele was relevant to a protective role for hepatocarcinogenesis. MTHFR rs9651118 was an intron SNP, which may influence the alternative splicing pattern. A functional study indicated that rs9651118 CC genotype of MTHFR, compared with TT genotype, reduced the Hcy level [48]. Recently, a dose–response meta-analysis concluded that each 5 μmol/l Hcy level promoting increased the incidence of digestive tract cancer by 7% [49]. Thus, MTHFR rs9651118 C allele may reduce the Hcy level, and then decrease the susceptibility of HCC.

Our findings suggested MTHFR rs1801133rs3753584rs4845882rs4846048rs9651118 increased the susceptibility of HCC. We first investigated the potential correlation of these MTHFR tagging SNPs with HCC susceptibility. It could be used as a potential biomarker for HCC diagnosis. Previous investigations have focused on the relationship between MTHFR haplotypes of these tagging SNPs and cancer susceptibility; however, MTHFR rs1801133rs3753584rs4845882rs4846048rs9651118 was not found to be associated with the risk of non-small cell lung cancer [50] and esophagogastric junction adenocarcinoma [47]. In the future, these findings should be further validated.

This hospital-based study might have some potential limitations. First, though we recruited 1507 subjects to investigate a relationship of MTHFR tagging SNPs with HCC, polymorphisms in other one-carbon metabolism genes were not included. Second, in the present study, we only included several risk factors (e.g., sex, chronic HBV infection, age, smoking and drinking), other environmental factors were not considered. Third, the present study was hospital-based, which could not fully represent the Chinese population and the bias might have happened. In the future, population-based investigations are needed to further explore the role of MTHFR SNPs to risk of HCC. Fourth, the intake of folate and diet habits were not collected in our study. Thus, we did not focus on the association of MTHFR variants and folate level with the susceptibility of HCC. Finally, we only evaluated the MTHFR SNPs with HCC, polymorphisms in other one-carbon metabolism genes were not included.

Taken together, in Chinese Han population, MTHFR rs9651118 and rs1801133 polymorphisms may be protective for HCC. However, MTHFR rs3753584 polymorphism is a candidate for susceptibility to HCC. In the future, these findings should be further validated in additional studies.
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Author Contribution

Conceived and designed the experiments: L.L. and W.T. Performed the experiments: S.Z., J.J. and J.L. Analyzed the data: Y.C. Contributed reagents/materials/analysis tools: S.Z., J.J. and Y.C. Wrote the manuscript: S.Z. and J.J.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

BC, breast cancer; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; Hcy, homocysteine; HWE, Hardy–Weinberg equilibrium; LC, liver cancer; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; SD, standard deviation; SNP, single nucleotide polymorphism; 5-MTHF, 5-methyltetrahydrofolate.

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