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RESEARCH ARTICLE

Genetic variations in the one-carbon metabolism pathway genes and susceptibility to hepatocellular carcinoma risk: a case–control study

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Abstract Hepatocellular carcinoma (HCC) is the sixth common cancer and the third common cause of cancer mortality worldwide. However, the exact molecular mechanism of HCC remains uncertain. Many enzymes are involved in one-carbon metabolism (OCM), and single nucleotide polymorphisms (SNPs) in the corresponding genes may play a role in liver carcinogenesis. In this study, we enrolled 1500 HCC patients and 1500 cancer-free controls, which were frequency-matched by age, gender, and HBV infection status. Then eight SNPs from seven OCM genes (MTHFR, MTR, MTRR, FTHFD, GART, SHMT, and CBS) were evaluated. Results showed that six SNPs (MTHFR rs1801133, MTRR rs2287780, MTRR rs10380, FTHFD rs1127717, GART rs8971, and SHMT rs1979277) were significantly associated with HCC risk in Chinese population, with P values range from 2.26×10^{-4} to 0.035). The most significant association was detected for GART rs8971. Compared with individuals with the TT genotype, the age- and sex-adjusted odds ratio (OR) for developing HCC was 1.44 (95 % confidence interval (CI): 1.03–2.02) among those with the CC genotype and 1.30 (95 % CI: 1.10–1.53) for those with CT genotype. Under the log-additive model, each additional copy of minor allele C was associated with a 1.28-fold increased risk of HCC (OR= 1.28, 95 % CI: 1.12–1.45). These findings indicated that genetic variants in OCM genes might contribute to HCC susceptibility.

Keywords Polymorphism · Hepatocellular carcinoma · One-carbon metabolism · Genetic · Case–control

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Introduction

Hepatocellular carcinoma (HCC), a primary malignancy of the liver, is now the third leading cause of cancer deaths worldwide, with over 500,000 people affected [1–3]. The epidemiology of HCC exhibits two main patterns, one in North America and Western Europe and another in non-Western countries, such as those in sub-Saharan Africa, central and Southeast Asia, and the Amazon basin [4–6]. In China, HCC is one of the most common cancer types, with an incidence rate of 53/100,000 per year and a death rate of 37–55/100,000 annually [6–8]. However, the etiology of HCC is still unknown, while some risk factors have been identified, such as alcoholism, hepatitis B, hepatitis C infection, aflatoxin, cirrhosis of the liver, hemochromatosis, genetic susceptibility, and so on [9–12].

There is evidence that deficiencies in nutrients involved in one-carbon metabolism (OCM), including folate and other nutrients, can cause impairment of immune responses and that immune deficiencies are known risk factors for HCC [13, 14]. Rats fed diets deficient in choline develop hepatocellular carcinoma, and tumor DNA from these animals is characteristically hypomethylated, suggesting that disruption of the OCM pathway is an underlying mechanism for hepatocarcinogenesis [13]. Considerable studies have specified that genetic variations, especially single nucleotide polymorphisms (SNPs), could contribute to HCC risk.

Through a comprehensive retrieval, we found that the research on the association of single nucleotide

polymorphisms (SNPs) on one-carbon metabolizing genes with risk of HCC to date have conflicting findings, and there are distinct differences between populations of different ethnic origin. In light of previous evidence, we hypothesized that potentially functional polymorphisms in key genes in OCM pathway may contribute to the carcinogenesis of HCC patients. Here, we screened a total of eight functional SNPs in seven genes (MTHFR, MTR, MTRR, FTHFD, GART, SHMT, and CBS) in the OCM pathway and examined their associations with risk of HCC in a Chinese population.

Materials and methods

Subjects

In the current study, cases were histopathologically confirmed HCC patients which were recruited at China Medical University from 2007, while controls without clinic evidence of hepatic diseases or tumors were randomly selected from a pool of healthy volunteers who visited the general health check-up center of the same hospital for routine scheduled physical exams. Controls were individually matched to cases on sex, ethnicity (Han), age (±3 years), and hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. After giving written consent, participants provided demographic information (including age, race, medical history for themselves and their families, food consumption history, and migration history) using a standard interviewer-administered questionnaire. Totally included in this study were 1500 cases and 1500 controls, representing 70 % of eligible cases and 20 % of eligible controls. Five milliliters of peripheral blood was obtained for DNA extraction. The present study was approved by the institutional review board of China Medical University (IRB-#031256). Written informed consent was given by all subjects, and collection of blood specimens was performed in accordance with the Declaration of Helsinki.

SNP selection and genotyping

We first searched for SNPs using SNP500 Cancer (http://snp500cancer.nci.nih.gov/). Then we identified and included SNPs that resulted in amino acid changes and were therefore potentially functional. Furthermore, the web tools, Polymorphism Phenotyping (PolyPhen), and Sorting Intolerant From Tolerant (SIFT), were used to predict the possible effect of each selected SNP on protein structure or function. Only potential damaging SNPs were recruited into this study. Finally, eight SNPs from seven genes (MTHFR, MTR, MTRR, FTHFD, GART, SHMT, and CBS) were selected. Genotyping was performed by using Illumina Golden Gate platform (Berkeley Biotech, Taizhou, China). Before genotyping, DNA quantity and quality were assessed using both fluorometer and agarose gel electrophoresis. Specific primer and probe sequences are available on request. A random 10 % of quality control samples were inserted in each genotyping run and concordance for these samples for each of the eight SNPs was 100 %. Laboratory personnel were blinded to the case–control status of the specimens and to the quality control samples.

Statistical analyses

All statistical analyses were conducted with SAS version 9.2 (SAS Institute Inc.). All statistical tests were two-tailed, and $P<0.05$ was interpreted as statistically significant unless otherwise indicated. Differences in the distribution of selected demographic variables between HCC cases and cancer-free controls were evaluated using the Student's t test for continuous variables or Pearson's χ^2 test for categorical variables. The deviation of the genotype frequencies of single nucleotide polymorphisms (SNPs) in the controls from those expected under the Hardy–Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's Chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the candidate genotypes between cases and controls. The associations between the polymorphisms and HCC risk were estimated by computing odds ratios (ORs) and their 95 % confidence intervals (CIs) from unconditional logistic regression analysis with the adjustment for possible confounders.

Results

Our final analysis included 1500 HCC cancer cases and 1500 healthy controls in this study (Table 1). There were no significant differences between cases and controls in terms of the distribution of age, sex, race, and HBV and HCV status, as these were individually matched.

Table 2 showed the genotypic frequencies for eight SNPs of OCM genes and their associations with HCC risk among Chinese Han people. None of the distribution of genotypes among control subjects deviate from the Hardy–Weinberg equilibrium ($P>0.05$). Among the eight SNPs investigated, the genotypic patterns of six SNPs (MTHFR rs1801133, MTRR rs2287780, MTRR rs10380, FTHFD rs1127717, GART rs8971, and SHMT rs1979277) were differentially distributed among the HCC patients and non-cancer controls, and the P value for trend was significant (P values range from 2.26×10^{-4} to 0.035). The most significant association was detected for GART rs8971. Compared with individuals with the TT genotype, the age- and sex-adjusted OR for developing HCC was 1.44 (95 % CI: 1.03–2.02) among those with the CC genotype and 1.30 (95 % CI: 1.10–1.53) for those with CT

Table 1 Demographic and etiologic characteristics of HCC cases and controls

	Cases (n=1500) No. (%)	Controls (n=1500) No. (%)	P value
Gender			
Male	1098 (73.2)	1121 (74.7)	
Female	402 (26.8)	379 (25.3)	0.339
Age			
≥50	765 (51.0)	783 (52.2)	
<50	735 (49.0)	717 (47.8)	0.511
HBV infection			
HBsAg (+)	1014 (67.9)	1004 (66.9)	
HBsAg (−)	486 (32.1)	496 (33.1)	0.697
HCV infection			
anti-HCV (+)	225 (15.0)	213 (14.2)	
anti-HCV (−)	1275 (85.0)	1287 (85.8)	0.535

genotype. Under the log-additive model, each additional copy of minor allele C was associated with a 1.28-fold increased risk of HCC (OR=1.28, 95 % CI: 1.12–1.45). Furthermore, the most strike effect was detected for MTRR rs10380. Compared with individuals with the CC genotype, those with the TT genotype have a higher HCC risk 1.50 (1.11–2.03) (OR= 1.50, 95 % CI: 1.11–2.03; P trend =0.008).

Besides, increased risk of HCC were also detected for MTHFR rs1801133, MTRR rs2287780, FTHFD rs1127717, and SHMT rs1979277. Compared with individuals with the wild homozygous genotype, those with the heterozygote genotype have a higher HCC risk (OR range from 1.15 to 1.18) and ORs for those with the heterozygote genotype range from 1.12 to 1.22. After adjusting for additional potentially confounding factors such as smoking status, alcohol consumption, body mass index, HBV infection status, and family history of cancer, the results did not change materially.

Discussion

In this large population-based case–control study, we analyzed eight non-synonymous SNPs in six genes that encode for enzymes involved in the OCM pathway, with the risk of HCC in a Chinese population. There was some suggestions that the variant alleles of six SNPs, including MTHFR rs1801133, MTRR rs2287780, MTRR rs10380, FTHFD rs1127717, GART rs8971, and SHMT rs1979277, were significantly associated with HCC risk. These provide evidence to implicate OCM gene polymorphisms as novel susceptibility factors for HCC risk.

MTHFR is a key regulatory enzyme in the folate metabolism, and MTHFR can catalyze 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which is the predominant circulating form of folate [15–17]. In addition, MTHFR also plays an important role in the folate metabolism by directing folate metabolites through the DNA methylation pathway [18–20]. Previous studies have suggested that MTHFR C677T polymorphism is associated with changes of plasma folate levels and the MTHFR activity [21, 22]. Currently, genetic polymorphisms of MTHFR gene are considered to have some influence on both folate metabolism and risks of many cancers, such as colorectal cancer, breast cancer, and cervical cancer, and our findings support the positive results of previous studies of the MTHFR rs1801133 and HCC risk [23–25].

In the current study, the most strike effect for HCC risk was detected for MTRR rs10380. MTR, a vitamin B12-dependent enzyme essential for maintaining adequate intracellular folate pools, catalyzes the remethylation of homocysteine to methionine [26]. MTRR maintains the activity of MTR by reduction reactions and transfers the methyl group of methyltetrahydrofolate to homocysteine via methionine synthase-methylcobalamin as an intermediate methyl carrier [27]. Studies have shown that genetic variants of MTRR were associated with many kinds of cancers [28–33]. Meanwhile, among the SNPs studied, GART rs8971 was detected as the most significant association for HCC risk in this study. GART gene is located in chromosome 21q22.11. The protein encoded by this gene is a trifunctional polypeptide, which has phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, and phosphoribosylaminoimidazole synthetase activity which is required for de novo purine biosynthesis [34–36]. All of our findings validated the importance of OCM genes in carcinogenesis of liver cell.

A strength of this study was the acceptable statistical power to distinguish relatively small genotype associations. Further larger-scale studies are warranted to examine gene–gene and gene–environment interactions on OCM pathway gene polymorphisms and HCC risk, which may ultimately lead to a comprehensive understanding of the conceivable roles in tumorgenesis. Also, some potential limitations of our study warrant discussion. First, this was not a comprehensive examination of 1-C metabolism genes. Second, we did not have information on dietary intake and could not verify which nutrient(s) was related to the modifying effects of multivitamins. Third, in spite of the relatively large sample size, the power to elucidate gene–environment interactions was limited because of the small magnitudes of the overall associations. Further studies should also include tagging SNPs and summarizing linkage equilibrium (LD) pattern within each gene in the OCM pathway. Pooling data from ongoing HCC studies will be required to confirm these findings.

In summary, data from this epidemiological study provide supportive evidence that OCM pathway underlies the development of HCC. Our finding warrants replication in a larger data set, and validations with larger population-based studies

Table 2 The associations between selected genetic polymorphisms in one-carbon metabolism pathway genes and risk of HCC

	Cases (*N*=1500)	Controls (*N*=1500)	Age- and sex-adjusted OR
MTHFR rs1801133			
CC	440 (29.3 %)	498 (33.2 %)	1.00 (reference)
CT	800 (53.3 %)	770 (51.3 %)	1.18 (1.00–1.38)
TT	260 (17.3 %)	232 (15.5 %)	1.27 (1.02–1.58)
T vs. C			1.12 (1.02–1.25)
P trend			*0.025*
MTR rs1805087			
AA	1260 (84.0 %)	1275 (85.0 %)	1.00 (reference)
AG	180 (12.0 %)	173 (11.5 %)	1.05 (0.84–1.32)
GG	60 (4.0 %)	52 (3.5 %)	1.17 (0.80–1.71)
G vs. A			1.09 (0.92–1.30)
P trend			0.314
MTRR rs2287780			
CC	1015 (67.7 %)	1065 (71.0 %)	1.00 (reference)
CT	373 (24.9 %)	338 (22.5 %)	1.16 (0.97–1.37)
TT	112 (7.4 %)	97 (6.5 %)	1.21 (0.91–1.61)
T vs. C			1.15 (1.01–1.31)
P trend			*0.032*
MTRR rs10380			
CC	990 (66.0 %)	1037 (69.1 %)	1.00 (reference)
CT	398 (26.5 %)	386 (25.7 %)	1.08 (0.92–1.27)
TT	112 (7.5 %)	77 (5.2 %)	1.50 (1.11–2.03)
T vs. C			1.19 (1.04–1.35)
P trend			*0.008*
FTHFD rs1127717			
TT	1112 (74.1 %)	1163 (77.5 %)	1.00 (reference)
CT	300 (20.0 %)	272 (18.2 %)	1.15 (0.96–1.38)
CC	88 (5.9 %)	65 (4.3)	1.43 (1.03–1.98)
C vs. T			1.22 (1.06–1.41)
P trend			*0.007*
GART rs8971			
TT	960 (64.0 %)	1052 (70.1 %)	1.00 (reference)
CT	455 (30.3 %)	383 (25.6 %)	1.30 (1.10–1.53)
CC	85 (5.7 %)	65 (4.3 %)	1.44 (1.03–2.02)
C vs. T			1.28 (1.12–1.45)
P trend			2.26×10^{-4}
SHMT rs1979277			
CC	1160 (77.3 %)	1200 (80.0 %)	1.00 (reference)
CT	242 (16.1 %)	218 (14.5 %)	1.15 (0.94–1.40)
TT	98 (6.6 %)	82 (5.5 %)	1.24 (0.91–1.67)
T vs. C			1.17 (1.01–1.36)
P trend			*0.035*
CBS rs706208			
CC	570 (38.1 %)	593 (39.5 %)	1.00 (reference)
CT	764 (50.9 %)	752 (50.1 %)	1.06 (0.90–1.23)
TT	166 (11.0 %)	155 (10.4)	1.11 (0.86–1.42)
T vs. C			1.05 (0.94–1.16)
P trend			0.382

P value in italics means statistically significant

in different ethnic groups, further research into the function of OCM pathway, and its potential biological mechanism association may be warranted. We believe that further study of the positive finding in Asian population may provide insight into the etiology of HCC.

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Conflicts of interest None

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