Methylenetetrahydrofolate reductase C677T (Ala>Val, rs1801133 C>T) polymorphism decreases the susceptibility of hepatocellular carcinoma: A meta-analysis involving 12,628 subjects

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Abstract

C677T (Ala>Val, rs1801133 C>T), a non-synonymous variant of methylenetetrahydrofolate reductase (MTHFR) gene, has been found to be associated with an impair enzyme activity of MTHFR. The relationship of MTHFR rs1801133 with hepatocellular carcinoma (HCC) has been extensively investigated. However, the findings were conflicting.Recently, more investigations have been conducted on the relationship of MTHFR rs1801133 with HCC. To obtain a more precise assessment on the effect of this non-synonymous variant to the development of HCC, a pooled-analysis was performed. This meta-analysis consisted of 19 independent case-control studies. By using the odds ratio (OR) combined with 95% confidence intervals (CIs), the relationship of MTHFR rs1801133 with HCC risk was determined. A total of nineteen independent case-control studies were included. Finally, 6,102 HCC cases and 6,526 controls were recruited to examine the relationship of MTHFR rs1801133 with HCC risk. In recessive model (TT vs. CC/CT), the findings reached statistical significance (OR, 0.90; 95%CI, 0.82-0.98; P=0.016). Subgroup analysis also found an association between MTHFR rs1801133 polymorphism and the decreased risk of HCC in hepatitis/virus related patients (recessive model: OR, 0.85; 95%CI, 0.72-0.99; P=0.035, and allele model: OR, 0.90; 95%CI, 0.81-0.99; P=0.028). Subgroup analyses indicated that extreme heterogeneity existed in Asian population, larger sample size investigation, hospital-based study and normal/healthy control subgroups. The shape of Begg’s test seemed symmetrical. Egger’s linear regression test also confirmed these evaluations. Sensitivity analyses suggested that our findings were stable. In summary, our results highlight that MTHFR rs1801133 polymorphism decreases HCC susceptibility. The relationship warrants a further assessment.
Introduction

In 2018, global cancer statistics estimated that liver malignancy was the fifth most frequent type of cancer incidence among men and the eleven most frequent type among women, about 596,574 and 244,506 new cases diagnosed worldwide, respectively [1]. However, the fatality was the third most frequent type [1]. The etiology of liver cancer (LC) was not well-established. Hepatocellular carcinoma (HCC) is one of the most important primary LC, which comprised almost 80% of LC cases. Some major susceptibility factors (e.g., aflatoxin-contaminated food, superabundant drinking, tobacco consumption, chronic virus infection, higher body mass index and type 2 diabetes) [2, 3, 4, 5, 6] may contribute to the development of HCC. Additionally, hereditary factor has also been suggested to affect the susceptibility for the occurrence of HCC.

Methylenetetrahydrofolate reductase (MTHFR) locates in 1p36.3, which maps from 11785723 to 11806103 (GRCh38; April, 2018). MTHFR, a key enzyme, plays a vital effect in folate metabolism by the role of catalyzing the 5,10-methylenetetrahydrofolate (5,10-methylene-THF) to 5-methyltetrahydrofolate (5-methylene-THF) irreversibly. In the conversion of homocysteine to methionine, 5-methylene-THF is a primary methyl donor [7]. MTHFR rs1801133 (C677T), a non-synonymous variant (Ala>Val), has been suggested to influence the activity of MTHFR enzyme [8]. The correlation of MTHFR rs1801133 polymorphism with malignancy has been extensively explored. This single nucleotide polymorphism (SNP) was suggested to be associated with thyroid cancer [9], colorectal cancer [10, 11], breast cancer [12], esophagogastric junction adenocarcinoma [13], non-small cell lung cancer [14], acute lymphoblastic leukemia [15, 16], gastric cancer [17], renal cell carcinoma [18], and esophageal carcinoma [19], among others.

Recently, many case-control studies have been carried out to determine the relationship of MTHFR rs1801133 polymorphism with the development of HCC [19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29]. However, the observations were controversial. Several meta-analyses also got conflicting results. To shed light on this issue, we conducted an extensive pooled-analysis to determine the role of MTHFR rs1801133 polymorphism on the development of HCC.

Materials and methods

Study searching

Publications were obtained by searching the PubMed and EMBASE databases before October 19, 2019. The following strategy was used: (Methylenetetrahydrofolate reductase OR MTHFR OR rs1801133) AND (SNP OR polymorphism) AND (cancer OR carcinoma) and (hepatocellular OR liver). The references in reviews and meta-analyses were also retrieved to get data. In this pooled-analysis, there was no language limited.

Inclusion Criteria

In our meta-analysis, the eligible criteria of the included publications were: (1) designed as a case-control study; (2) focusing on the relationship of the MTHFR rs1801133 polymorphism with HCC risk; (3) genotype data could be extracted and (4)
publications were compatible with Hardy–Weinberg equilibrium (HWE) in controls.

**Exclusion Criteria**

The criteria for exclusion were as following: (1) publications incompatible with HWE; (2) overlapping data; (3) not case-control study design and (4) only focusing on the relationship of MTHFR rs1801133 polymorphism with HCC survival.

**Data extraction**

The authors (S. Zhang and J. Jiang) extracted the following data: the surname of first author, publication year, populations studied, country where the investigation was carried out, ratio of sex, age, positive (%) of hepatitis B surface antigen (HBsAg), genotyping method, the number of participants and MTHFR rs1801133 genotype. If there was conflicting assessment, another reviewer (W. Tang) was invited. During this process, they made a vote to obtain the final decision.

**Statistical methods**

In this study, the odds ratio (OR) combined with 95% confidence intervals (CIs) were harnessed to compare the difference between HCC group and controls. P value (<0.05) was considered statistically significant. The present meta-analysis determined the correlation in four genetic models [e.g. dominant model (TT/CT vs. CC), homzygote model (TT vs. CC), allele model (T vs. C) and recessive model (TT vs. CC/CT)]. Using $I^2$ metric and Q statistic, the heterogeneity among the eligible case-control studies was evaluated. If $I^2$<0.10 or $I^2$>50%, we defined that there was significant heterogeneity. Thus, the random-effect model was used [30, 31]. Otherwise, there was no heterogeneity detected. A fixed-effect model was used to combine the data [32]. The Egger test and Begg's test were used to assess the bias of publication. If $P$<0.10, we defined that there was a significant publication bias. By omitting a study one by one and analyzing the remainders, sensitivity analysis was performed to assess the stability of our findings. The distribution of the MTHFR rs1801133 genotype was used to calculate the $P$ value of HWE by using an online software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) in controls [33, 34, 35]. STATA 12.0 software (Stata Corp., College Station, Texas) was used to conduct the analysis. In this study, $P$ value was two sided.

**Quality assessment of meta-analysis**

Two authors (SZ and JJ) independently extracted the data and calculated the quality score of the included case-control studies. The detailed scores were determined by a quality assessment criteria, which were presented in previous studies [36, 37]. If the scores were more than 6.0, the investigation had acceptable quality [38].

**Results**

**Eligible studies**

A total of eleven publications were eligible (Figure 1). Four articles involved several different subgroups, so we considered them as independent investigations. After a screening, nineteen independent case-control studies were included. In addition, five publications were excluded for incompatible with HWE [29, 39, 40, 41, 42]. Finally, 6,102 HCC cases and 6,526 controls were recruited to examine the
relationship of MTHFR rs1801133 polymorphism with HCC risk (Table 1). The publication year covered from 2004 to 2019. These investigations were performed in different populations: three were conducted in mixed populations [28, 29], four were carried out in Caucasians [26, 27], and twelve involved Asians [19, 20, 21, 22, 23, 24, 25]. The MTHFR rs1801133 genotypes are summarized in Table 2.

**Meta-analysis results**

In the eligible investigations, the MAF of MTHFR rs1801133 C/T polymorphism was 0.473 in HCC patients (5,670/11,944) and was 0.466 in controls (5,721/12,288). In different race, the MAF of controls was not similar in controls. The MAFs were 0.352 (480/1362) in mixed populations, 0.328 (214/652) in Caucasians, and that was 0.485 (5,075/10,462) in Asians.

The pooled-analysis findings were reported in four genetic models including 19 independent case-control studies. In recessive model (TT vs. CC/CT), the findings reached statistical significance (OR, 0.90; 95%CI, 0.82-0.98; P=0.016; Table 3 and Figure 2). In other genetic models, we failed to obtain the significance (dominant model: OR, 0.92; 95%CI, 0.81-1.05; P=0.209, homozygote model: OR, 0.88; 95%CI, 0.77-1.01; P=0.078, and allele model: OR, 0.93; 95%CI, 0.85-1.01; P=0.077, Table 3).

Subgroup analyses were carried out according to the following terms: ethnicity (Caucasians or Asians or mixed), sample sizes (<1,000 or ≥1,000 subjects), control type [normal/healthy subjects or hepatitis/virus related patients or not available (NA)] and source of control [hospital-based (HB) or population-based (PB) or NA]. We pooled seven case-control studies (including 2,435 HCC cases and 1,642 hepatitis/virus related patients) and found an association between MTHFR rs1801133 polymorphism and decreased risk of HCC in hepatitis/virus related patients (recessive model: OR, 0.85; 95%CI, 0.72-0.99; P=0.035, and allele model: OR, 0.90; 95%CI, 0.81-0.99; P=0.028, Table 3). When we conducted a subgroup analysis by ethnicity, null association between MTHFR rs1801133 C>T polymorphism and the risk of HCC was found.

**Heterogeneity assessment**

In some genetic models, heterogeneity was significant (Table 3). Subgroup analyses indicated that extreme heterogeneity existed in Asian populations, larger sample size investigation, HB study and normal/healthy control subgroups. If we excluded subgroups in our meta-analysis, the heterogeneity significantly decreased.

**Bias evaluation**

We used Begger’s and Egger’s tests to identify the bias of publication among the included investigations. The shape of Begger’s test seemed symmetrical. Egger’s linear regression test also confirmed these evaluations (Figure 3).

**Sensitivity Analyses**

By sequentially omitting an individual investigation, sensitivity analysis was carried out. This method is considered as a criterion for meta-analysis. The results indicated that the significance of this study could not be altered by removing any case-control study (Figure 4), suggesting that our findings were stable.

**Quality assessment**
Table 2 presents the results of the quality evaluation. Each eligible study had an acceptable quality (scores ≥6).

**Discussion**

Accumulating investigations highlight that **MTHFR** rs1801133 polymorphism may be associated with the development of HCC. However, the findings of the previous case-control studies were conflicting, with several investigations suggesting a potential relationship, whereas others did not support the correlation. In this investigation, to explore whether the **MTHFR** rs1801133 polymorphism was implicated in the etiology of HCC, we carried out a pooled-analysis of nineteen eligible studies, which recruited 6,102 HCC cases and 6,526 controls. This meta-analysis indicated that the **MTHFR** rs1801133 polymorphism was a protective factor for the development of HCC in the overall comparison. Compared to the previous study, this pooled-analysis first confirmed the association of **MTHFR** rs1801133 polymorphism with a decreased risk of HCC.

**MTHFR** rs1801133 polymorphism locates on 11796321 (NCBI Build 38) of Chromosome 1. Zhu and her/his colleagues first reported that **MTHFR** rs1801133 polymorphism might confer a risk to HCC [24]. In addition, Cui et al. also suggested that this polymorphism could increase the risk of HCC [20]. However, some case-control studies indicated that the rs1801133 polymorphism in **MTHFR** gene might decrease the susceptibility of HCC [21, 25]. And most studies reported that this SNP in **MTHFR** gene could not alter the risk of HCC. Thus, the association of **MTHFR** rs1801133 polymorphism with the susceptibility of HCC was more conflicting. Here, we performed a pooled-analysis of nineteen eligible studies involving 6,102 HCC cases and 6,526 controls to explore the correlation of rs1801133 with the etiology of HCC. The results indicated that this SNP in **MTHFR** gene could be a protective factor to an occurrence of HCC. Two meta-analyses suggested that rs1801133 was not associated with HCC development [43, 44]. Others pooled-analyses reported that **MTHFR** rs1801133 polymorphism was associated with an increased risk of HCC [45, 46, 47, 48]. Compared to these early meta-analyses, our analysis included more large sample size studies [21, 25]. It is worth mentioning that these more recent case-control studies have recruited more participants and reported that rs1801133 polymorphism was a protective factor for the development of HCC. Compared to the most recent meta-analysis [23], the merit of our study was the larger sample size and the detailed subgroup analysis. Combined the eligible studies, we observed that rs1801133 decreased the susceptibility of HCC in the overall comparison. The quality score was evaluated in our study. Each eligible study had an acceptable quality (scores ≥6). This indicated that our findings were reliable. We also found an association between **MTHFR** rs1801133 polymorphism and decreased risk of HCC in hepatitis/virus related patients. Of late, in Asian population, some meta-analyses identified that **MTHFR** rs1801133 polymorphism decreased the risk of colorectal cancer [49, 50]. Some publications [51, 52] suggested that **MTHFR** rs1801133 C>T polymorphism (Ala→Val) could promote the level of 5,10-methylene-THF for DNA synthesis, which might be protective to carcinogenesis.
In the future, a functional study should be carried out to address how this Ala→Val substitution could decrease the risk of HCC.

Heterogeneity was identified in the overall comparison. In this study, we conducted subgroup analyses to explore the major source among the eligible studies. Subgroup analysis suggested that major heterogeneity might be due to different populations, sample size, and characteristics of controls.

Some potential limitations should be addressed in this pooled-analysis. Firstly, only published investigations were eligible in our study. Thus, the number of included case-control studies might be inadequate. Secondly, for lacking of sufficient data, only crude ORs and CIs were calculated. Thirdly, the controls in some of the case-control studies were hepatitis or virus related patients. Fourthly, a recent investigation contained some subgroups, we treated them as independent case-control studies. However, in this literature, the same HCC group was used in different stratified analysis. Finally, our study did not focus on the gene-gene and gene-environment interactions.

In summary, the present pooled-analysis highlights that MTHFR rs1801133 polymorphism was a protective factor for the occurrence of HCC, especially in hepatitis/virus related patients. The relationship of MTHFR rs1801133 polymorphism with HCC risk warrants a further determination.

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Author contribution statement:
Conceived and designed the experiments: SZ, LL
Performed the experiments: SZ, JJ
 Analyzed the data: WT, SZ
 Contributed reagents/materials/analysis tools: LL
 Wrote the manuscript: SZ, JJ
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References:

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018 Nov;68(6):394-424. doi: 10.3322/caac.21492. PubMed PMID: 30207593.

2. Pinero F, Pages J, Marciano S, et al. Fatty liver disease, an emerging etiology of hepatocellular carcinoma in Argentina. World journal of hepatology. 2018 Jan 27;10(1):41-50. doi: 10.4254/wjh.v10.i1.41. PubMed PMID: 29399277; PubMed Central PMCID: PMC5787683.

3. Liew ZH, Goh GB, Hao Y, et al. Comparison of Hepatocellular Carcinoma in Patients with Cryptogenic Versus Hepatitis B Etiology: A Study of 1079 Cases Over 3 Decades. Digestive diseases and sciences. 2018 Oct 16. doi: 10.1007/s10620-018-5331-x. PubMed PMID: 30327962.

4. Ioannou GN, Green P, Lowy E, et al. Differences in hepatocellular carcinoma risk, predictors and trends over time according to etiology of cirrhosis. PloS one. 2018;13(9):e0204412. doi: 10.1371/journal.pone.0204412. PubMed PMID: 30260995; PubMed Central PMCID: PMC6160079.

5. Jaquet A, Tchounga B, Tanon A, et al. Etiology of hepatocellular carcinoma in West Africa, a case-control study. International journal of cancer. 2018 Aug 15;143(4):869-877. doi: 10.1002/ijc.31393. PubMed PMID: 29569722; PubMed Central PMCID: PMC6041181.

6. Ghouri YA, Mian I, Rowe JH. Review of hepatocellular carcinoma: Epidemiology, etiology, and carcinogenesis. Journal of carcinogenesis. 2017;16:1. doi: 10.4103/jcar.JCar_9_16. PubMed PMID: 28497440; PubMed Central PMCID: PMC5490340.

7. Kopp M, Morisset R, Rychlik M. Characterization and Interrelations of One-Carbon Metabolites in Tissues, Erythrocytes, and Plasma in Mice with Dietary Induced Folate Deficiency. Nutrients. 2017 May 05;9(5). doi: 10.3390/nu9050462. PubMed PMID: 28475162.

8. Jain M, Pandey P, Tiwary NK, et al. MTHFR C677T polymorphism is associated with hyperlipidemia in women with polycystic ovary syndrome. Journal of human reproductive sciences. 2012 Jan;5(1):52-6. doi: 10.4103/0974-1208.97802. PubMed PMID: 22870016; PubMed Central PMCID: PMC3409921.

9. Zara-Lopes T, Galbiatti-Dias ALS, Castanhole-Nunes MMU, et al. Polymorphisms in MTHFR, MTR, RFC1 and CssS genes involved in folate metabolism and thyroid cancer: a case-control study. Archives of medical science : AMS. 2019 Mar;15(2):522-530. doi: 10.5114/aoms.2018.73091. PubMed PMID: 30899306; PubMed Central PMCID: PMC6425207.

10. Lin KM, Yang MD, Tsai CW, et al. The Role of MTHFR Genotype in Colorectal Cancer Susceptibility in Taiwan. Anticancer research. 2018 Apr;38(4):2001-2006. doi: 10.21873/anticancer.12438. PubMed PMID: 29599316.

11. Zhang S, Chen S, Chen Y, et al. Investigation of methylenetetrahydrofolate reductase tagging polymorphisms with colorectal cancer in Chinese Han population. Oncotarget. 2017 Sep 8;8(38):63518-63527. doi: 10.18632/oncotarget.18845. PubMed PMID: 28969008; PubMed Central PMCID: PMC5609940.

12. Hesari A, Maleksabet A, Tirkani AN, et al. Evaluation of the two polymorphisms rs1801133 in MTHFR and rs10811661 in CDKN2A/B in breast cancer. Journal of cellular biochemistry.
13. Ding G, Wang Y, Chen Y, et al. Methylenetetrahydrofolate reductase tagging polymorphisms are associated with risk of esophagogastric junction adenocarcinoma: a case-control study involving 2,740 Chinese Han subjects. Oncotarget. 2017 Dec 19;8(67):111482-111494. doi: 10.18632/oncotarget.22845. PubMed PMID: 29299150; PubMed Central PMCID: PMC5762337.

14. Ding H, Wang Y, Chen Y, et al. Methylenetetrahydrofolate reductase tagging polymorphisms are associated with risk of non-small cell lung cancer in eastern Chinese Han population. Oncotarget. 2017 Dec 15;8(66):110326-110336. doi: 10.18632/oncotarget.22887. PubMed PMID: 29299150; PubMed Central PMCID: PMC5762337.

15. Xia X, Duan Y, Cui J, et al. Association of methylenetetrahydrofolate reductase gene-gene interaction and haplotype with susceptibility to acute lymphoblastic leukemia in Chinese children. Leukemia & lymphoma. 2017 Aug;58(8):1887-1892. doi: 10.1080/10428194.2016.1265117. PubMed PMID: 27996344.

16. Ramirez-Pacheco A, Moreno-Guerrero S, Alamillo I, et al. Mexican Childhood Acute Lymphoblastic Leukemia: A Pilot Study of the MDR1 and MTHFR Gene Polymorphisms and Their Associations with Clinical Outcomes. Genetic testing and molecular biomarkers. 2016 Oct;20(10):597-602. doi: 10.1089/gtmb.2015.0287. PubMed PMID: 27533339.

17. Wei L, Niu F, Wu J, et al. Association study between genetic polymorphisms in folate metabolism and gastric cancer susceptibility in Chinese Han population: A case-control study. Molecular genetics & genomic medicine. 2019 May;7(5):e633. doi: 10.1002/mgge.3.633. PubMed PMID: 30884202; PubMed Central PMCID: PMC6503009.

18. Lv C, Bai Z, Liu Z, et al. Renal cell carcinoma risk is associated with the interactions of APOE, VHL and MTHFR gene polymorphisms. International journal of clinical and experimental pathology. 2015;8(5):5781-6. PubMed PMID: 26191297; PubMed Central PMCID: PMC4503168.

19. Chang SC, Chang PY, Butler B, et al. Single nucleotide polymorphisms of one-carbon metabolism and cancers of the esophagus, stomach, and liver in a Chinese population. PloS one. 2014;9(10):e109235. doi: 10.1371/journal.pone.0109235. PubMed PMID: 25337902; PubMed Central PMCID: PMC4206280.

20. Cui LH, Song Y, Si H, et al. Folate metabolism-related gene polymorphisms and susceptibility to primary liver cancer in North China. Medical oncology. 2012 Sep-Oct;29(3):1837-42. doi: 10.1007/s12032-011-0066-y. PubMed PMID: 21956592.

21. Jiao X, Luo Y, Yang B, et al. The MTHFR C677T mutation is not a risk factor recognized for HBV-related HCC in a population with a high prevalence of this genetic marker. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases. 2017 Apr;49:66-72. doi: 10.1016/j.meegid.2017.01.008. PubMed PMID: 28082187.

22. Kwak SY, Kim UK, Cho HJ, et al. Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) gene polymorphisms as risk factors for hepatocellular carcinoma in a Korean population. Anticancer research. 2008 Sep-Oct;28(5A):2807-11. PubMed PMID: 19035314.

23. Su H. Correlation Between MTHFR Polymorphisms and Hepatocellular Carcinoma: A Meta-analysis. Nutrition and cancer. 2019;71(7):1055-1060. doi:
24. Zhu ZZ, Cong WM, Liu SF, et al. [A study on the association of MTHFR C677T polymorphism with genetic susceptibility to hepatocellular carcinoma]. Zhonghua gan zang bing za zhi = Zhonghua ganzangbing zazhi = Chinese journal of hepatology. 2006 Mar;14(3):196-8. PubMed PMID: 16556414.

25. Zhang S, Lin J, Jiang J, et al. Association between methylenetetrahydrofolate reductase tagging polymorphisms and susceptibility of hepatocellular carcinoma: a case-control study. Bioscience reports. 2019 Nov 29;39(11). doi: 10.1042/BSR20192517. PubMed PMID: 31694048; PubMed Central PMCID: PMC6852349.

26. Fabris C, Toniutto P, Falleti E, et al. MTHFR C677T polymorphism and risk of HCC in patients with liver cirrhosis: role of male gender and alcohol consumption. Alcoholism, clinical and experimental research. 2009 Jan;33(1):102-7. doi: 10.1111/j.1530-0277.2008.00816.x. PubMed PMID: 18945219.

27. Saffroy R, Pham P, Chiappini F, et al. The MTHFR 677C > T polymorphism is associated with an increased risk of hepatocellular carcinoma in patients with alcoholic cirrhosis. Carcinogenesis. 2004 Aug;25(8):1443-8. doi: 10.1093/carcin/bgh147. PubMed PMID: 15033905.

28. Peres NP, Galbiatti-Dias AL, Castanhole-Nunes MM, et al. Polymorphisms of folate metabolism genes in patients with cirrhosis and hepatocellular carcinoma. World journal of hepatology. 2016 Oct 18;8(29):1234-1243. doi: 10.4254/wjh.v8.i29.1234. PubMed PMID: 27803768; PubMed Central PMCID: PMC5067443.
36. Thakkinstian A, McEvoy M, Minelli C, et al. Systematic review and meta-analysis of the association between beta2-adrenoeceptor polymorphisms and asthma: a HuGE review. American journal of epidemiology. 2005 Aug 1;162(3):201-11. doi: 10.1093/aje/kwi184. PubMed PMID: 15987731.

37. Camargo MC, Mera R, Correa P, et al. Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006 Sep;15(9):1674-87. doi: 10.1158/1055-9965.EPI-06-0189. PubMed PMID: 16985030.

38. Guo J, Jin M, Zhang M, et al. A genetic variant in miR-196a2 increased digestive system cancer risks: a meta-analysis of 15 case-control studies. PloS one. 2012;7(1):e30585. doi: 10.1371/journal.pone.0030585. PubMed PMID: 22291993; PubMed Central PMCID: PMC3265498.

39. D’Amico M, Pasta L, Sammarco P. MTHFR C677TT, PAI1 4G-4G, V Leiden Q506, and prothrombin G20210A in hepatocellular carcinoma with and without portal vein thrombosis. Journal of thrombosis and thrombolysis. 2009 Jul;28(1):70-3. doi: 10.1007/s11239-008-0246-6. PubMed PMID: 18618228.

40. Qiao K, Zhang S, Trieu C, et al. Genetic Polymorphism of MTHFR C677T Influences Susceptibility to HBV-Related Hepatocellular Carcinoma in a Chinese Population: a Case-Control Study. Clinical laboratory. 2017 Apr 1;63(4):787-795. doi: 10.7754/Clin.Lab.2016.161003. PubMed PMID: 28397480.

41. Wang C, Xie H, Lu D, et al. The MTHFR polymorphism affect the susceptibility of HCC and the prognosis of HCC liver transplantation. Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico. 2018 Apr;20(4):448-456. doi: 10.1007/s12094-017-1729-8. PubMed PMID: 29185200.

42. Zhang H, Liu C, Han YC, et al. Genetic variations in the one-carbon metabolism pathway genes and susceptibility to hepatocellular carcinoma risk: a case-control study. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2015 Feb;36(2):997-1002. doi: 10.1007/s13277-014-2725-z. PubMed PMID: 25318605.

43. Su H, Zhang G. Correlation between Methylene tetrahydrofolate Reductase Polymorphisms and Hepatocellular Carcinoma: A Meta-Analysis. Annals of nutrition & metabolism. 2019;74(3):251-256. doi: 10.1159/000496428. PubMed PMID: 30917367.

44. Qin X, Peng Q, Chen Z, et al. The association between MTHFR gene polymorphisms and hepatocellular carcinoma risk: a meta-analysis. PloS one. 2013;8(2):e56070. doi: 10.1371/journal.pone.0056070. PubMed PMID: 23457501; PubMed Central PMCID: PMC3573065.

45. Jin F, Qu LS, Shen XZ. Association between the methylenetetrahydrofolate reductase C677T polymorphism and hepatocellular carcinoma risk: a meta-analysis. Diagnostic pathology. 2009 Nov 24;4:39. doi: 10.1186/1746-1596-4-39. PubMed PMID: 19930673; PubMed Central PMCID: PMC2788519.

46. Qi X, Sun X, Xu J, et al. Associations between methylenetetrahydrofolate reductase polymorphisms and hepatocellular carcinoma risk in Chinese population. Tumour biology :
47. Qi YH, Yao LP, Cui GB, et al. Meta-analysis of MTHFR C677T and A1298C gene polymorphisms: association with the risk of hepatocellular carcinoma. Clinics and research in hepatology and gastroenterology. 2014 Apr;38(2):172-80. doi: 10.1007/s13277-013-1023-5. PubMed PMID: 24316043.

48. Sun H, Han B, Zhai H, et al. Significant association between MTHFR C677T polymorphism and hepatocellular carcinoma risk: a meta-analysis. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014 Jan;35(1):189-93. doi: 10.1007/s13277-013-1023-5. PubMed PMID: 24132589.

49. Teng Z, Wang L, Cai S, et al. The 677C>T (rs1801133) polymorphism in the MTHFR gene contributes to colorectal cancer risk: a meta-analysis based on 71 research studies. PloS one. 2013;8(2):e55332. doi: 10.1371/journal.pone.0055332. PubMed PMID: 23437053; PubMed Central PMCID: PMC3577825.

50. Guo XP, Wang Y, Zhao H, et al. Association of MTHFR C677T polymorphisms and colorectal cancer risk in Asians: evidence of 12,255 subjects. Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico. 2014 Jul;16(7):623-9. doi: 10.1007/s12094-013-1126-x. PubMed PMID: 24193867.

51. Taioli E, Garza MA, Ahn YO, et al. Meta-and pooled analyses of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and colorectal cancer: a HuGE-GSEC review. American journal of epidemiology. 2009 Nov 15;170(10):1207-21. doi: 10.1093/aje/kwp275. PubMed PMID: 19846566; PubMed Central PMCID: PMC2781761.

52. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature genetics. 1995 May;10(1):111-3. doi: 10.1038/ng0595-111. PubMed PMID: 7647779.
Figure legends:

Figure 1. Flow diagram of this meta-analysis

Figure 2. Meta-analysis of the association between MTHFR rs1801133 polymorphism and HCC risk (recessive model, fixed-effects model)

Figure 3. Begg’s funnel plot of meta-analysis of the association between MTHFR rs1801133 polymorphism and HCC risk (recessive model, fixed-effects model)

Figure 4. Sensitivity analysis of the influence of MTHFR rs1801133 polymorphism to HCC risk (recessive model, fixed-effects model)
Table 1. Characteristics of the studies in meta-analysis

| Study | Year | Sample size | Country | Ethnicity | Sex, male (%) | Age (years); Case/control | Drinking (%); Case/control | HBsAg, positive (%) | Genotype method | Source of control | Type of control |
|-------|------|-------------|---------|-----------|---------------|--------------------------|----------------------------|---------------------|-----------------|-----------------|----------------|
| Cui   | 2012 | 356/641     | China   | Asian     | 83.1/43.5     | 56.6/58.7               | 44.1/30.3                 | 77.5/8.6            | Real-time PCR  | PB              | Normal or healthy control |
| Fabris| 2009 | 65/147      | Italy   | Caucasian | NA/NA         | NA/NA                   | NA                        | 26.2/10.9           | PCR-RFLP       | HB              | Hepatitis or virus related control |
| Fabris| 2009 | 65/236      | Italy   | Caucasian | NA/69.5       | NA/48                   | NA                        | 89.1/0.0            | PCR-RFLP       | HB              | NA              |
| Jiao  | 2017 | 726/549     | China   | Asian     | 72.7/54.6     | 56.5/41.5               | 24.5/NA                   | 89.1/100            | TaqMan         | HB              | Normal or healthy control |
| Jiao  | 2017 | 726/558     | China   | Asian     | 72.7/53.6     | 56.5/33.7               | 24.5/NA                   | 89.1/0.0            | TaqMan         | HB              | Hepatitis or virus related control |
| Jiao  | 2017 | 726/242     | China   | Asian     | 72.7/66.5     | 56.5/39.6               | 24.5/13.6                 | 89.1/100            | TaqMan         | HB              | Hepatitis or virus related control |
| Jiao  | 2017 | 726/704     | China   | Asian     | 72.7/64.9     | 56.5/53.7               | 24.5/23.6                 | 89.1/88.7           | TaqMan         | HB              | Hepatitis or virus related control |
| Kwak  | 2008 | 96/201      | Korea   | Asian     | NA             | 57.6/53.6               | NA                        | NA                  | PCR-RFLP       | HB              | Normal or healthy control |
| Peres | 2016 | 71/356      | Brazil  | Mixed     | 73.2/73.3     | NA/NA                   | NA                        | 62.0/46.0           | PCR-RFLP       | HB              | Normal or healthy control |
| Peres | 2016 | 71/116      | Brazil  | Mixed     | 73.2/74.1     | NA/62.0                 | NA                        | 62.0/53.4           | PCR-RFLP       | HB              | Hepatitis or virus related control |
| Saffroy| 2004 | 72/122     | France  | Caucasian | 84.7/85.2     | 55/50                   | NA                        | NA                  | PCR-RFLP       | HB              | Hepatitis or virus related control |
| Saffroy| 2004 | 27/80      | France  | Caucasian | 74.1/86.3     | 54/54                   | NA                        | NA                  | PCR-RFLP       | HB              | Normal or healthy control |
| Saffroy| 2004 | 49/30      | France  | Caucasian | 85.7/66.7     | 56/52                   | NA                        | NA                  | PCR-RFLP       | HB              | Hepatitis or virus related control |
| Xu    | 2014 | 205/200     | China   | Asian     | NA             | 52.0/61.0               | NA                        | NA                  | PCR            | NA              | NA              |
| Yuan  | 2007 | 118/209     | USA     | Mixed     | 68.6/61.2     | NA/71.2                 | 68.4                      | 28.0/11.5           | TaqMan         | PB              | Normal or healthy control |
| Zhu   | 2006 | 508/543     | China   | Asian     | 85.8/48.8     | 50/45                   | 39.8/17.9                 | 72.8/17.9           | PCR-RFLP       | HB              | Normal or healthy control |
| Chang | 2014 | 204/415     | China   | Asian     | 77.9/69.2     | 53.9/57.7               | 41.7/35.7                 | 64.7/24.6           | PCR-RFLP       | PB              | Normal or healthy control |
| Zhang | 2019 | 584/923     | China   | Asian     | 89.9/90.5     | 53.2/53.7               | 29.1/16.0                 | 70.6/9.2            | SNPscan        | HB              | Normal or healthy control |

PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism

PCR: polymerase chain reaction

SNP: single-nucleotide polymorphism

HP: hospital-based
PB: population-based

NA: not available
Table 2. Distribution of *MTHFR* rs1801133 C>T polymorphism genotype and allele

| Study  | Year | Case TT | Case CT | Case CC | Control TT | Control CT | Control CC | case T | case C | control T | control C | HWE | Quality assessment |
|--------|------|---------|---------|---------|------------|------------|------------|--------|--------|-----------|-----------|-----|-------------------|
| Cui    | 2012 | 125     | 179     | 52      | 195        | 121        | 429        | 283    | 715    | 567       |           | Yes | 7.0               |
| Fabris | 2009 | 13      | -       | CC/CT=52| 23         | CC/CT=124  | -          | -      | -      | -         | -         | Yes | 6.5               |
| Fabris | 2009 | 13      | -       | CC/CT=52| 54         | 69         | -          | -      | -      | -         | -         | Yes | 6.5               |
| Jiao   | 2017 | 188     | 370     | 168     | 176        | 263        | 110        | 746    | 706    | 615       | 483       | Yes | 7.5               |
| Jiao   | 2017 | 188     | 370     | 168     | 169        | 268        | 121        | 746    | 706    | 606       | 510       | Yes | 7.5               |
| Jiao   | 2017 | 188     | 370     | 168     | 29         | 35         | 17         | 746    | 706    | 93        | 69        | Yes | 6.5               |
| Jiao   | 2017 | 188     | 370     | 168     | 120        | 222        | 100        | 746    | 706    | 462       | 422       | Yes | 7.5               |
| Jiao   | 2017 | 188     | 370     | 168     | 215        | 338        | 151        | 746    | 706    | 768       | 640       | Yes | 7.5               |
| Kwak   | 2008 | 18      | 46      | 32      | 31         | 106        | 64         | 82     | 110    | 168       | 234       | Yes | 6.5               |
| Peres  | 2016 | 7       | 36      | 28      | 33         | 174        | 149        | 50     | 92     | 240       | 472       | Yes | 7.0               |
| Peres  | 2016 | 7       | 36      | 28      | 13         | 55         | 48         | 50     | 92     | 81        | 151       | Yes | 6.0               |
| Saffroy| 2004 | 5       | 24      | 43      | 10         | 60         | 52         | 34     | 110    | 80        | 164       | Yes | 6.5               |
| Saffroy| 2004 | 2       | 16      | 9       | 13         | 37         | 30         | 20     | 34     | 63        | 97        | Yes | 6.5               |
| Saffroy| 2004 | 5       | 29      | 15      | 3          | 17         | 10         | 39     | 59     | 23        | 37        | Yes | 6.5               |
| Xu     | 2014 | 50      | 112     | 43      | 50         | 111        | 39         | 212    | 198    | 211       | 189       | Yes | 6.5               |
| Yuan   | 2007 | 14      | 51      | 53      | 30         | 99         | 80         | 79     | 157    | 159       | 259       | Yes | 7.0               |
| Zhu    | 2006 | 110     | 226     | 172     | 102        | 268        | 173        | 446    | 570    | 472       | 614       | Yes | 8.0               |
| Chang  | 2014 | 30      | 114     | 50      | 57         | 199        | 135        | 174    | 214    | 313       | 469       | Yes | 7.5               |
| Zhang  | 2019 | 49      | 227     | 299     | 103        | 446        | 372        | 325    | 825    | 652       | 1190      | Yes | 8.0               |

HWE: Hardy–Weinberg equilibrium.
### Table 3. Results of the meta-analysis from different comparative genetic models

| No. of studies | Ethnicity | Sample sizes | Source of control | Control type |
|---------------|-----------|--------------|------------------|--------------|
|               |           | <1,000       | P-B              | Normal or healthy |
| 19            | Asia      | 6            | 3,3           | 10            |
| 5             | Caucasians| 1            | 1              | 7             |
| 3             | Mixed     | 1            | NA             | 2             |

|                  | P         | T         |     |     |     |     |     |     |     |     |     | P         | T         |     |     |     |     |     |     |     |     |     |     | P         | T         |
|                  | OR(95% CI) | F         |     |     |     |     |     |     |     |     |     | OR(95% CI) | F         |     |     |     |     |     |     |     |     |     |     | OR(95% CI) | F         |     |
|                  | P         | I²        |     |     |     |     |     |     |     |     |     | P         | I²        |     |     |     |     |     |     |     |     |     |     | P         | I²        |     |
|                  | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |
|                  | OR(95% CI) | (Q-test)  |     |     |     |     |     |     |     |     |     | OR(95% CI) | (Q-test)  |     |     |     |     |     |     |     |     |     |     | OR(95% CI) | (Q-test)  |     |
|                  | P         | I²        |     |     |     |     |     |     |     |     |     | P         | I²        |     |     |     |     |     |     |     |     |     |     | P         | I²        |     |
|                  | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |
|                  | OR(95% CI) | (Q-test)  |     |     |     |     |     |     |     |     |     | OR(95% CI) | (Q-test)  |     |     |     |     |     |     |     |     |     |     | OR(95% CI) | (Q-test)  |     |
|                  | P         | I²        |     |     |     |     |     |     |     |     |     | P         | I²        |     |     |     |     |     |     |     |     |     |     | P         | I²        |     |
|                  | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |
|                  | OR(95% CI) | (Q-test)  |     |     |     |     |     |     |     |     |     | OR(95% CI) | (Q-test)  |     |     |     |     |     |     |     |     |     |     | OR(95% CI) | (Q-test)  |     |
|                  | P         | I²        |     |     |     |     |     |     |     |     |     | P         | I²        |     |     |     |     |     |     |     |     |     |     | P         | I²        |     |
|                  | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |
|                  | OR(95% CI) | (Q-test)  |     |     |     |     |     |     |     |     |     | OR(95% CI) | (Q-test)  |     |     |     |     |     |     |     |     |     |     | OR(95% CI) | (Q-test)  |     |
|                  | P         | I²        |     |     |     |     |     |     |     |     |     | P         | I²        |     |     |     |     |     |     |     |     |     |     | P         | I²        |     |
|                  | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |
|                  | OR(95% CI) | (Q-test)  |     |     |     |     |     |     |     |     |     | OR(95% CI) | (Q-test)  |     |     |     |     |     |     |     |     |     |     | OR(95% CI) | (Q-test)  |     |
|                  | P         | I²        |     |     |     |     |     |     |     |     |     | P         | I²        |     |     |     |     |     |     |     |     |     |     | P         | I²        |     |
|                  | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |     |     |     |     |     |     |     |     |     | (Q-test)  | (Q-test)  |     |

### Notes:
- **P-B**: population-based;
- **H-B**: hospital-based;
- **NA**: not available
Publications extracted from PubMed and Embase databases (n=266)

Two hundred and nine articles regarding the association of MTHFR rs1801133 polymorphism with HCC risk

Two hundred and forty-two articles were excluded (one hundred and eighty-three were uncorrelated to the relationship between MTHFR rs1801133 and HCC risk, nine were reviews and/or meta-analyses, three focused on the prognosis and treatment, one were designed as not case-control study and one was duplicated data).

Twelve articles screened

Manual search of the reference lists of articles (n=3)

Twenty-four independent case-control studies in fifteen articles were identified.

Finally, nineteen independent case-control studies in eleven articles focusing on the relationship between MTHFR rs1801133 polymorphism and HCC risk were eligible.

Five independent case-control studies excluded for violation with HWE.
| Study ID    | OR (95% CI) | Weight |
|------------|-------------|--------|
| Caucasian  |             |        |
| Saffroy (2004) | 0.84 (0.27, 2.55) | 0.68   |
| Saffroy (2004) | 0.41 (0.09, 1.96) | 0.60   |
| Saffroy (2004) | 1.02 (0.23, 4.63) | 0.33   |
| Fabris (2009) | 1.35 (0.63, 2.86) | 1.12   |
| Fabris (2009) | 0.84 (0.42, 1.65) | 1.85   |
| Subtotal (I-squared = 0.0%, p = 0.712) | 0.92 (0.61, 1.40) | 4.58   |
| Asian      |             |        |
| Zhu (2006)  | 1.19 (0.88, 1.62) | 7.63   |
| Kwak (2008) | 1.27 (0.67, 2.40) | 1.61   |
| Cui (2012)  | 1.24 (0.94, 1.63) | 8.93   |
| Xu (2014)   | 0.97 (0.62, 1.52) | 3.78   |
| Chang (2014)| 1.07 (0.66, 1.73) | 3.16   |
| Jiao (2017) | 0.74 (0.58, 0.95) | 14.68  |
| Jiao (2017) | 0.80 (0.63, 1.03) | 14.00  |
| Jiao (2017) | 0.63 (0.39, 1.02) | 3.82   |
| Jiao (2017) | 0.94 (0.72, 1.23) | 10.92  |
| Jiao (2017) | 0.79 (0.63, 1.00) | 15.99  |
| Zhang (2019)| 0.74 (0.52, 1.06) | 7.16   |
| Subtotal (I-squared = 44.4%, p = 0.055) | 0.89 (0.81, 0.98) | 91.68  |
| Mixed      |             |        |
| Yuan (2007) | 0.80 (0.41, 1.58) | 1.89   |
| Peres (2016)| 1.07 (0.45, 2.53) | 0.98   |
| Peres (2016)| 0.87 (0.33, 2.29) | 0.88   |
| Subtotal (I-squared = 0.0%, p = 0.874) | 0.89 (0.56, 1.42) | 3.74   |
| Overall (I-squared = 11.9%, p = 0.309) | 0.90 (0.82, 0.98) | 100.00 |
Begg’s funnel plot with pseudo 95% confidence limits
