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Original Article

Association between *MTHFR* A1298C polymorphism and hepatocellular carcinoma risk

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Abstract: Background: Hepatocarcinogenesis is a complex process that is influenced by many factors. Several studies have investigated the relationship between *MTHFR* A1298C polymorphism and hepatocellular carcinoma (HCC) risk, but the results are inconsistent. Therefore, we performed a meta-analysis covering a large sample size to address this controversy. Methods: Eligible studies were searched using PubMed, EMBASE, and China National Knowledge Infrastructure (CNKI) databases. A total of 7 studies from 6 publications with 2035 cases and 3096 controls were included. The pooled odds ratios (ORs) with 95% confidence intervals (CIs) was calculated by the fixed or random effects to evaluate the correlation between *MTHFR* A1298C polymorphism and HCC risk. The Q statistic and I^2 statistic were used to assess the statistical heterogeneity among studies. Publication bias was evaluated by Egger's linear regression test and Begg's funnel plot. Results: In present study, the results showed that *MTHFR* A1298C polymorphism was not significantly associated with risk of HCC based on CC + AC vs. AA genetic model (OR=1.01, 95% CI=0.90-1.13). Similarly, in the subgroup analysis by ethnicity, no significant HCC risk was found in Asian population (OR=1.02, 95% CI=0.91-1.14). In the subgroup analysis based on source of control, we found that *MTHFR* A1298C polymorphism showed no effects on the occurrence of HCC in the population-based (PB) and hospital-based (HB) group (OR=0.97, 95% CI=0.83-1.15; OR=1.04, 95% CI=0.89-1.21). Conclusion: This meta-analysis suggested that *MTHFR* A1298C polymorphism may not be a risk factor for HCC.

Keywords: Hepatocellular carcinoma, *MTHFR,* polymorphism

Introduction

Hepatocellular carcinoma (HCC), one of the most malignant tumors, is particularly induced by unregulated growth and metastasis. It is the third leading cause of cancer death due to easy metastasis and frequent recurrence [1-5]. The development of HCC was effected by genetic and environmental factors. Hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol drinking and cigarette smoking were reported to play vital roles in the occurrence of HCC [6, 7], but the effects will be shown only under certain genetic background. So genes are the necessary element for the development and progress of HCC. Moreover, recent studies showed that many genes play important roles in HCC development, such as *MTHFR*, *ADAMTS5*, *IFNL3, IL-12* and so on [8-11].

Methylenetetrahydrofolate reductase (*MTHFR*) gene decodes a rate-limiting enzyme in the methyl cycle, which is located in chromosome 1p36.3 in human, containing 14 exons [12, 13]. One function of *MTHFR* enzyme is to catalyze the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate [14]. It is well known that *MTHFR* gene is involved in both DNA methylation and DNA synthesis and many studies have suggested that *MTHFR* serves as a potential risk factor for liver cancer in patients with chronic liver disease. In addition, genetic variations of *MTHFR* have been reported to be associated with the gene deficiency and may influence individual susceptibility to occlusive vascular disease [15], neural tube defects [16], Alzheimer's disease [17] and other forms of dementia, colon cancer, and acute leukemia [18, 19].

Although many studies about the relationship of *MTHFR* A1298C polymorphism with HCC were carried out, the results were controversial. To derive a more precise estimation of the relationship both of two, we conducted a meta-analysis of all available case-control studies.

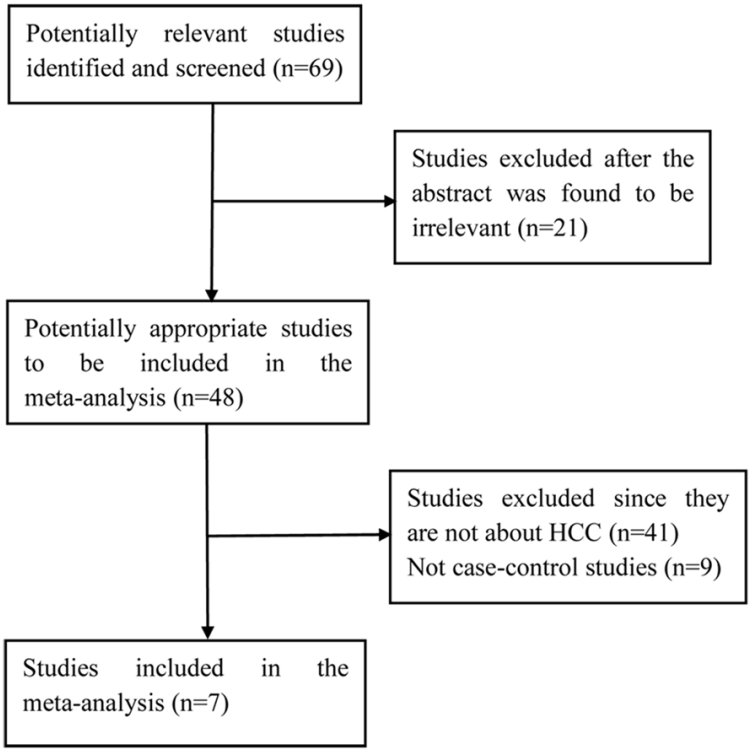

Figure 1. Flow chart of study selection.

Table 1. Summary of eligible studies considered in the meta-analysis

First author	Year	Country	Ethnicity	Control source	Genotyping method	Case	Control	HWE
Cui	2012	China	Asian	PB	RT-PCR	356	641	0.00
Yuan 2	2007	America	Mixed	PB	TaqMan	118	209	0.67
Yuan 1	2007	China	Asian	PB	TaqMan	247	248	0.31
Kwak	2008	Korea	Asian	HB	PCR-RFLP	96	201	0.26
Mu	2007	China	Asian	PB	PCR-RFLP	194	394	0.25
Yang	2007	China	Asian	HB	PCR-RFLP	327	185	
An	2008	China	Asian	HB	TaqMan	697	1218	0.72

Note: PCR: polymerase chain reaction; RT-PCR: Real time PCR; PCR-RFLP: PCR-restriction fragment length polymorphism; TaqMan: TaqManSNP; HWE: Hardy-Weinberg equilibrium.

Materials and methods

Search strategy

All case-control studies of *MTHFR* A1298C polymorphism and HCC risk were identified through systematic searches in PubMed, EMBASE, and CNKI databases. The search terms were: "methylenetetrahydrofolate reductase", "*MTHFR*", "polymorphism", "genetic variations" "mutant" "mutation", "hepatocellular carcinoma", "HCC", "liver cancer", "liver tumor", "liver neoplasms" and "hepatic tumor". For each article identified, manual search of the relevant references was also performed. The search was performed without language limitation.

Inclusion criteria

The study was included in the meta-analysis if it satisfied the following criteria: (1) it assessed the correlation between HCC and the *MTHFR* A1298C polymorphism; (2) it had a case-control design; (3) it provided sufficient genotype data for estimating an odds ratio (OR) with 95% confidence interval (95% CI); (4) it studied on human beings. In case of multiple studies with the same or overlapping data published by same researchers, we selected the most recent study with the largest number of participants. Studies were excluded if: (1) the design was a family-based; (2) the genotype frequency was not reported.

Data extraction

Data extraction was carried out independently by authors. For each study, the following characteristics were noted: the first author's name, year of publication, country of origin, ethnicity, numbers and genotypes of cases and controls, source of controls (hospital-based or population-based) and genotyping method. Discrepancies were resolved by a discussion.

Statistical analysis

The genotypes distribution of *MTHFR* A1298C in the control population was checked by Hardy Weinberg Equilibrium (HWE). The pooled OR and 95% CI were calculated as the indicators to assess the relationship between *MTHFR*

Table 2. Subgroup analysis of *MTHFR* A1298C polymorphism and HCC risk by ethnicity and source of control

		CC + AC vs. AA	
		OR (95% CI)	P_h
Ethnicity	Asian	1.02 (0.91, 1.14)	0.957
	Mixed	0.89 (0.60, 1.33)	-
Source of control	PB	0.97 (0.83, 1.15)	0.973
	HB	1.04 (0.89, 1.21)	0.636
Total		1.01 (0.90, 1.13)	0.963

Note: Ph: *P*-value for heterogeneity test; OR: odds ratio; CI: confidence interval; PB: population-based; HB: hospital-based.

A1298C polymorphism and HCC susceptibility. The Q and I^2 statistic were used to assess the statistical heterogeneity among studies. If $P<0.05$ or $I^2 \geq 50\%$, which indicated the presence of heterogeneity, a random-effect model was used to estimate the OR; otherwise, a fixed-effect model was used. Z test was employed to evaluate the significance of ORs with 95% CI. Publication bias was evaluated by Egger's test [20] and Begg's funnel plot. Sensitivity analysis was performed by sequential omission of individual studies. All results were analyzed by STATA 12.0 (Stata Corporation, College Station, TX, USA). All *P* values were two-sided test and the significant level was set at 0.05.

Results

Study characteristics

A total of 69 articles were retrieved after searching the databases, of which 21 were excluded because the abstract was irrelevant, and 41 was excluded since they did not focus on hepatocellular carcinoma. As shown in **Figure 1**, after the detailed selection, 7 case-control studies fulfilled the inclusion criteria and were included in the meta-analysis [8, 21-25]. A summary about the information extracted from each article was listed in **Table 1**.

Meta-analysis result

The pooled OR with 95% CI were analyzed under the genetic model of CC + AC vs. AA (**Table 2**). The outcome suggested that the *MTHFR* A1298C polymorphism was not significantly associated with HCC risk (OR=1.01, 95% CI=0.90-1.13, *P*=0.963) in the overall analysis.

Stratified analysis

As shown in **Figures 2** and **3**, no significant association between *MTHFR* A1298C polymorphism with risk of HCC was found in neither Asian population nor mixed population (Asian population: CC + AC vs. AA: OR=1.02, 95% CI=0.91-1.14, *P*=0.957; mixed population: CC + AC vs. AA: OR=0.89, 95% CI=0.60-1.33). The same results were applied to hospital-based (HB) control and population-based (PB) control (HB: CC + AC vs. AA: OR=0.89, 95% CI=0.60-1.33; PB: CC + AC vs. AA: OR=0.97, 95% CI=0.83-1.15).

Heterogeneity test

In the present study, we used the Q and I^2 test to evaluate the heterogeneity across studies. As shown in **Table 2**, there was no significant heterogeneity ($P > 0.05$).

Sensitivity analysis

Sensitivity analysis was carried out to evaluate the effect of a single data-set on the pooled ORs. The corresponding pooled ORs were not materially altered after deleting one data-set (data not shown), indicating that our results were statistically robust.

Publication bias

Both Begg's and Egger's tests were used to evaluate the publication bias of the 7 studies. The shape of the funnel plots did not reveal any evidence of obvious asymmetry for the genetic model in the meta-analysis (**Figure 4**). Moreover, Egger's test did not present any significantly statistical evidence of publication bias (*P*=0.982).

Discussion

HCC is the sixth most common cancer and the third leading cause of cancer-related deaths worldwide [26]. In the previous studies, hepatitis B, hepatitis C, heavy alcohol consumption, tobacco smoking, and diabetes were proved as risk factors for HCC [27, 28]. Like any other cancer, the genetic mutations functioning in cell cycle and apoptosis will promote the development of HCC. But rapid progression and diffi-

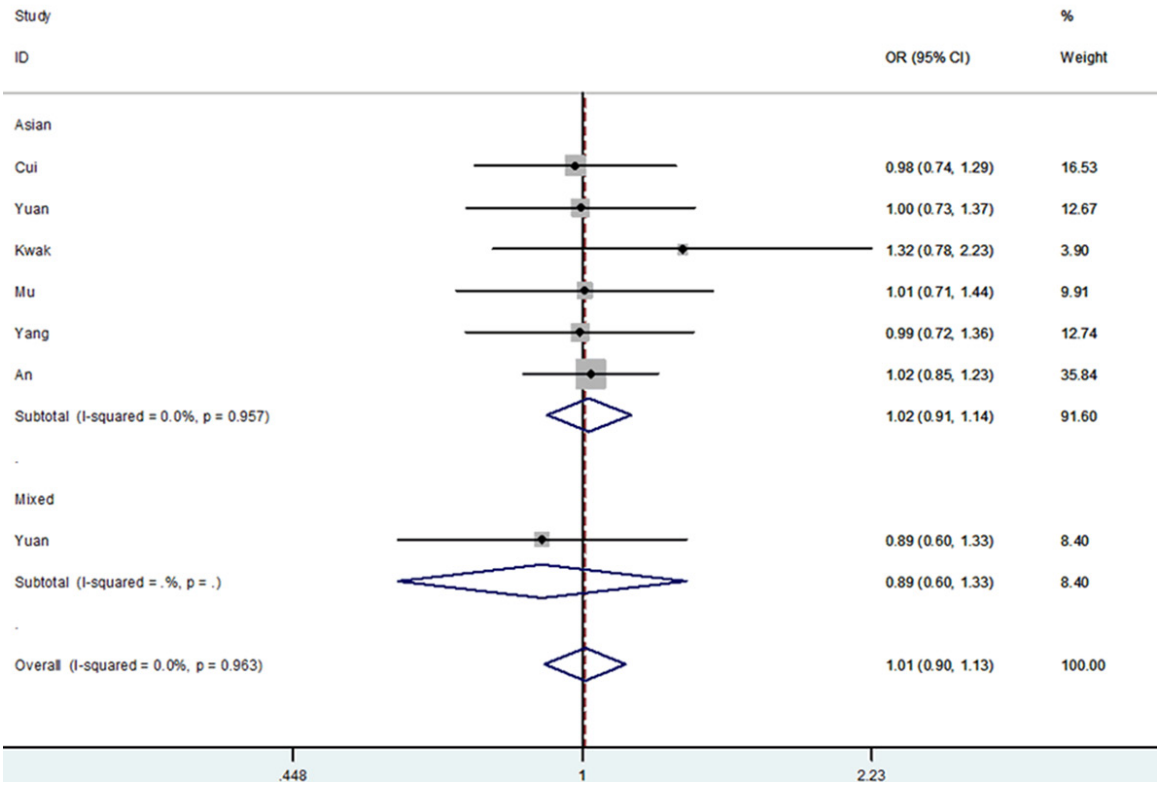

Figure 2. Forest plots illustrating the subgroup analysis by ethnicity.

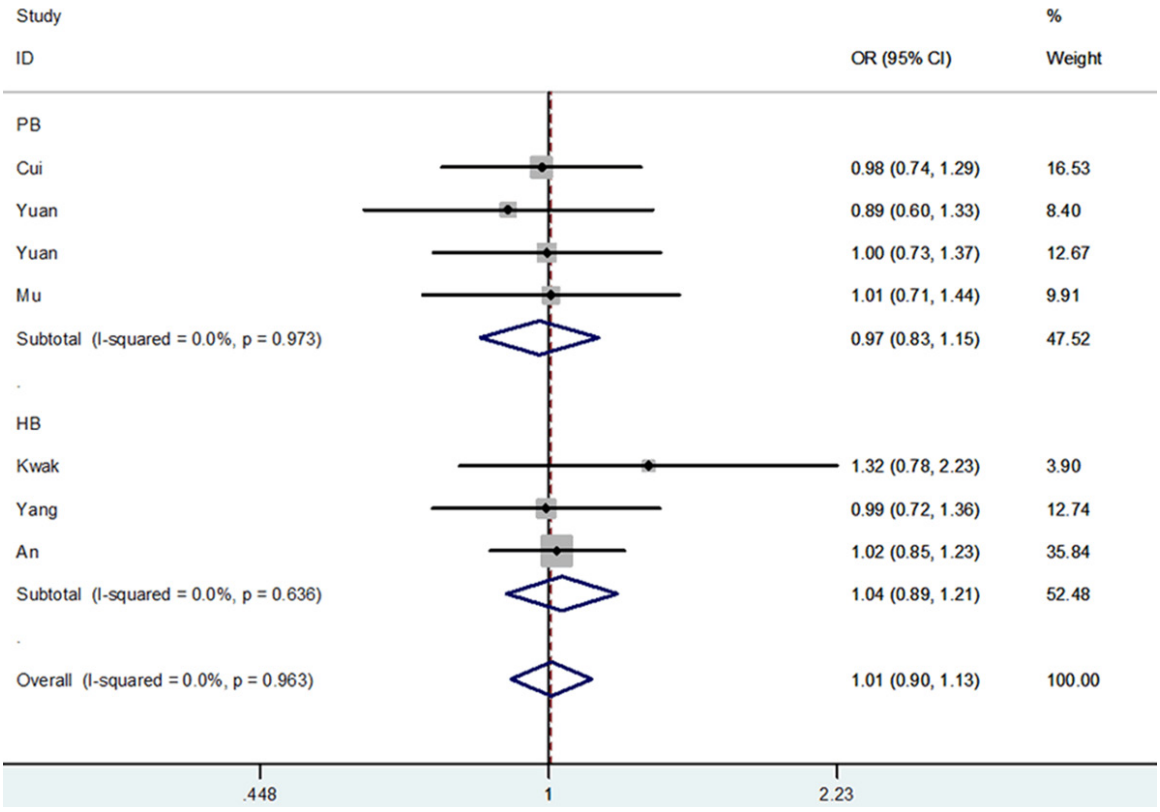

Figure 3. Forest plots illustrating the subgroup analysis by source of control.

Figure 4. Funnel plot analysis to detect publication bias.

culty in early diagnosing are the major obstacles for the treatment of HCC [29]. So our meta-analysis was aimed to obtain precise estimations on the relationship of *MTHFR* A1298C polymorphism with HCC, which will help in the early diagnosis and therapy of HCC.

It has been concluded that *MTHFR* plays an essential role in DNA synthesis and methylation [30, 31]. Genetic variations of *MTHFR* maybe result in some organs diseased, such as breast, stomach, lung, especially in the liver [8, 32-34]. The study of Saffroy R et al. showed that the mutant homozygous genotype CC of *MTHFR* 677C > T polymorphism significantly increased the risk of HCC in patients who have a heavy drinking habit [31]. However, *MTHFR* G1793A heterozygote has a significant reduced risk for liver cancer in those who gained folate supplements [35]. Recently, two meta-analyses were performed to assess the correct relationship of *MTHFR* A1298C polymorphism with HCC susceptibility, both results showed that the former was a protective factor for the latter [36, 37]. But our meta-analysis suggested that there was no association between *MTHFR* A1298C polymorphism and HCC risk, so are the subgroup analyses of ethnicity and source of control. The diverse results maybe should attribute to different inclusion criteria and sample size.

Some limitations of this meta-analysis should be considered when interpreting the results. First, cancer is a multi-factorial disease caused by environmental and genetic factors. Some environmental factors, however, may predominate in the development of cancer, such as living habits and exposure to carcinogens. While our analysis only focused on the roles of genetic mutant in the development of HCC, which might influence the accuracy on the result. Second, lacking of the original data limited our further evaluation for potential gene-environment interactions. Moreover, the published studies included in our meta-analysis provided no sufficient sample size for a comprehensive analysis.

In conclusion, this meta-analysis suggested that the *MTHFR* A1298C polymorphism might not be associated with the risk of HCC. Further large and well-design analyses are needed to validate this correlation. Moreover, gene-gene and gene-environment interactions should also be considered.

Disclosure of conflict of interest

None.

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