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Significant association between the MTHFR A1298C polymorphism and hepatocellular carcinoma risk: a meta-analysis

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ABSTRACT. The A1298C polymorphism of the methylenetetrahydrofolate reductase (*MTHFR*) gene has been reported to be associated with hepatocellular carcinoma (HCC), but there are conflicting results from previous studies. The present study aimed to investigate the association between this polymorphism and the risk of HCC using a meta-analysis of the published studies. Published literature from PubMed and Embase databases was systematically searched to identify relevant studies before October 2014. The Begg test was used to measure publication bias. Sensitivity analyses were performed to ensure the authenticity of the outcome. The meta-analysis results showed significant association between the MTHFR A1298C polymorphism and HCC risk (CC *vs* AA: OR = 0.52, 95%CI = 0.33-0.81; CC *vs* AC: OR = 0.50, 95%CI = 0.32-0.79; dominant model: OR = 1.94, 95%CI = 1.24-3.02; recessive model: OR = 1.00, 95%CI = 0.84-1.18). In the subgroup analysis, significant associations

between the MTHFR A1298C polymorphism and HCC risk were found in Asians (CC *vs* AA: OR = 0.46, 95%CI = 0.27-0.78; CC *vs* AC: OR = 0.41, 95%CI = 0.24-0.71; dominant model: OR = 2.27, 95%CI = 1.33-3.86; recessive model: OR = 1.03, 95%CI = 0.86-1.24). Our results suggest that the MTHFR A1298C polymorphism might be related to increased risk of HCC in Asians. Further large and well-designed studies are needed to confirm these conclusions.

Key words: Hepatocellular carcinoma; A1298C polymorphism; Methylenetetrahydrofolate reductase (MTHFR)

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third largest cause of cancer-related deaths worldwide (Greten et al., 2008). Owing to the fact that most HCC patients at the advanced stage are prone to present a poor prognosis, early HCC detection is urgently needed. Chronic hepatitis B virus and hepatitis C virus infections are the major cause of HCC (El-Serag, 2012). In addition, other well-established risk factors for HCC include smoking tobacco, alcohol consumption, and aflatoxin exposure (Koh et al., 2011; Liu et al., 2012; Lin et al., 2013). However, only a minority of individuals who are at risk will develop HCC, indicating that the conventional risk factors can only partly explain the pathogenesis of HCC. In the past two decades, more and more studies have found that some variants in human genes are associated with HCC, indicating that genetic background also plays a role in hepatocellular carcinogenesis.

Folate is a water-soluble vitamin that is naturally found in green leafy vegetables, cereals, legumes, and fruit. Deficiency of folate can induce defective DNA repair and fragile chromosomal site expression, leading to chromosomal breaks and micronucleus formation (Aune et al., 2011). Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the metabolism of folate (Guenther et al., 1999). It catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the primary circulating form of folate and provides methyl groups for the methylation of homocysteine to methionine, the precursor of S-adenosyl methionine (SAMe) (Rosenblatt, 2001). SAMe is the principal biological methyl donor for methylation of DNA and other large molecules, and SAMe deficiency can predispose the patient to HCC. Altered MTHFR enzyme activity has been linked to the development of HCC (Lu and Mato, 2005).

The *MTHFR* gene has the chromosomal locus 1p36.3, is 2.2 kb long, and has a total of 11 exons (Goyette et al., 1998). Two common functional polymorphisms have been identified in the *MTHFR* gene: the MTHFR C677T polymorphism (the 677C→T transition leads to an alanine to valine substitution); and the A1298C polymorphism (the 1298A→C transition leads to a glutamic acid to alanine substitution). These polymorphisms lead to a 30-60% reduction in MTHFR enzyme activity (Frosst et al., 1995; van der Put et al., 1998). Recent meta-analyses have suggested that the MTHFR C677T polymorphism is associated with an increased risk of HCC in Asians (Sun et al., 2014).

In the past decade, a number of case-control studies have evaluated the association between the MTHFR A1298C polymorphism and HCC risk. However, the results have been inconsistent or even contradictory. This is partially owing to the possibly small effect of the polymorphism on cancer risk and the relatively small sample size in each published study. Meta-

analysis is a powerful tool for summarizing the different studies. Not only can it overcome the problem of small size and inadequate statistical power of genetic studies of complex traits, but it can also provide more reliable results than a single case-control study. To further shed light on the influence of the MTHFR A1298C polymorphism on HCC risk, a meta-analysis was performed of all available case-control studies.

MATERIAL AND METHODS

Selection of studies

Eligible studies were identified by searching PubMed and Embase databases for relevant reports before October 2014 using the following search terms: “folate or MTHFR”, “polymorphism or polymorphisms” and “hepatocellular carcinoma (HCC)”. There was no language limitation. The retrieved studies were manually screened to ensure they complied with the eligibility criteria. We reviewed abstracts of all citations and retrieved relevant studies.

Inclusion and exclusion criteria

The following inclusion criteria were applied: 1) the studies must have evaluated the association between the MTHFR A1298C polymorphism and HCC; 2) the studies must have had a case-control design; 3) the papers must have offered the size of the samples, the distribution of alleles, the genotypes, or other information that could help us interpret the results to estimate the odds ratios (ORs) and their 95% confidence intervals (CIs); and 4) the most recent studies were chosen if studies had overlapping patients or controls. The following exclusion criteria were applied: 1) the design of the study was based on family or sibling pairs, 2) the genotype frequency was not reported, or 3) there was insufficient information for data extraction.

Data extraction

The following information was extracted from each study: first author’s surname, year of publication, ethnicity of study population, country where the study was conducted, genotyping method, the number of cases and controls for each A1298C genotype, and whether the gene distribution of the controls was in compliance with Hardy-Weinberg equilibrium (HWE). When articles included subjects of more than one ethnicity, data were extracted separately for future subgroup analysis. Data were independently extracted by two reviewers using a standardized data extraction form, and discrepancies were resolved by discussion.

Quality score assessment

The quality of these studies was also evaluated independently by the same two investigators according to the predefined quality assessment rules in Table 1 (Jiang et al., 2010). The criteria cover the representativeness of cases, source of controls, confirmation of ovarian cancer, total sample size, quality control of genotyping methods, and HWE in the control population. Disagreements were resolved by consensus. The total scores ranged from 0 (worst) to 15 (best). Papers scoring <10 were classified as “low quality” and those scoring ≥10 as “high quality.”

Table 1. Scale for quality assessment.

Criteria	Score
Source of cases	
Selected from population or cancer registry	3
Selected from hospital	2
Selected from pathology archives, but without description	1
Not described	0
Source of controls	
Population-based	3
Blood donors or volunteers 2	2
Hospital-based (cancer-free patients) 1	1
Not described 0	0
Specimens of cases determining genotypes	
White blood cells or normal tissues	3
Tumor tissues or exfoliated cells of tissue	0
Hardy-Weinberg equilibrium in controls	
Hardy-Weinberg equilibrium	3
Hardy-Weinberg disequilibrium	0
Total sample size	
≥1000	3
≥500 but <1000	2
≥200 but <500	1
>0 but <200	0

Statistical analysis

The pooled ORs and 95%CIs were calculated as the indicators to assess the relationship between the MTHFR A1298C polymorphisms and HCC susceptibility under a co-dominant model (CC *vs* AA, CC *vs* AC), a dominant model (AA + AC *vs* CC), and a recessive model (CC + AC *vs* AA). The chi-square test was used to investigate HWE in the genotypes in a control group of each study included. Between-study heterogeneities were estimated using the I^2 test (Higgins et al., 2003). I^2 represents the variability that can be attributed to heterogeneity rather than chance. I^2 values of 25, 50, and 75% were defined as low, moderate, and high estimates, respectively. When a significant I^2 > 50% indicated heterogeneity across studies, the random-effect model was used for meta-analysis, otherwise the fixed-effect model was used. At the same time, subgroup analyses were conducted by stratification of ethnicity. Sensitivity analysis was performed by comparing the random-effect model values with the fixed-effect model values. Publication bias was investigated by the Begg test funnel plot ($P < 0.05$ was considered representative of statistically significant publication bias). All the statistical tests were performed with STATA version 12.0 (Stata Corporation, College Station, TX, USA). All P values are two-sided.

RESULTS

Characteristics of the studies included

The search strategy retrieved 25 potentially relevant studies. Based on the inclusion criteria, five case-control studies from four publications were included in this meta-analysis (Mu et al., 2007; Yuan et al., 2007; Kwak et al., 2008; Cui et al., 2012) and 21 studies were excluded. The flow chart for the study selection is summarized in Figure 1. The five studies selected included a total of 1011 cases and 1690 healthy controls. The year of publication of the studies included ranged from 2004 to 2014. All five eligible studies were case-control studies; four were of a population-

based design, and the remaining study was hospital-based (Kwak et al., 2008). All the articles were written in English. Four of the studies were of Asians and one study was of Caucasians (Yuan et al., 2007). All studies included were of high quality as the quality score assessment of each one was higher than or equal to 10 points, and the genotype distributions in all controls were consistent with HWE except one study (Cui et al., 2012). General characteristics and the allele and genotype distributions in the published articles included in this meta-analysis are shown in Table 2.

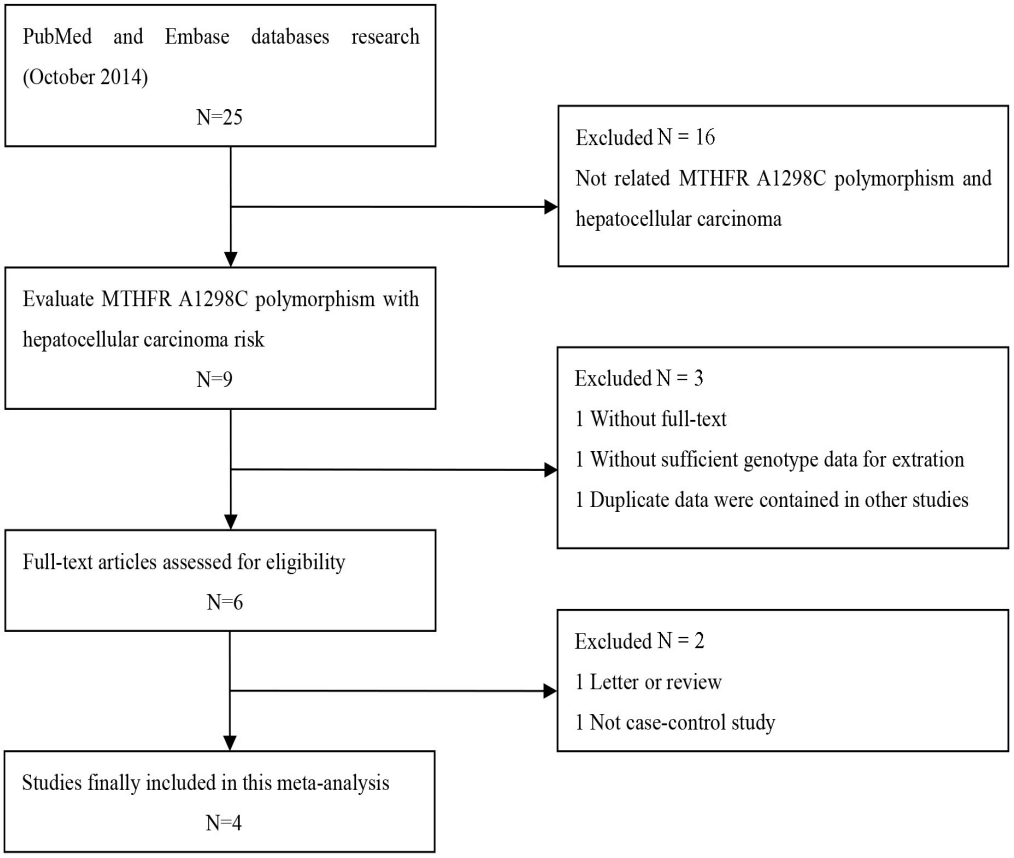

Figure 1. Flow chart of study selection based on the inclusion and exclusion criteria.

Table 2. Characteristics of the studies included for meta-analysis.

Study	Year	Area	Race	Source of controls	Cases/Controls	Genotypes for cases			Genotypes for controls			HWE test	Quality scores
						AA	AC	CC	AA	AC	CC		
Mu et al.	2007	China	Asians	PB	194/391	135	55	4	275	112	7	0.25	14
Yuan et al. A	2007	USA	Caucasians	PB	118/209	65	44	9	104	85	20	0.67	10
Yuan et al. B	2007	China	Asians	PB	247/248	136	101	10	136	91	21	0.31	11
Kwak et al.	2008	Korea	Asians	HB	96/201	67	28	1	155	41	5	0.26	10
Cui et al.	2012	China	Asians	PB	356/641	258	94	4	461	153	27	0.00	10

PB, population-based; HB, hospital-based; HWE, Hardy-Weinberg equilibrium.

Quantitative synthesis

A summary of the meta-analysis findings of the association between the MTHFR A1298C polymorphism and HCC risk is shown in Figure 2 and Table 3. The combined results based on all studies showed that variant genotypes are associated with increased HCC risk in the different genetic models (CC *vs* AA: OR = 0.52, 95%CI = 0.33-0.81; CC *vs* AC: OR = 0.50, 95%CI = 0.32-0.79; the dominant model: OR = 1.94, 95%CI = 1.24-3.02; the recessive model: OR = 1.00, 95%CI = 0.84-1.18). Sensitivity analyses were conducted by altering the statistical models. No material alteration was detected, indicating that our results were statistically robust.

Considering the potential impact of the confounding factors on the overall results, we further performed subgroup analyses. In the primary literature, only the detailed information on ethnicity and the source of controls were sufficient for analysis. Hence, subgroup analyses on these issues were carried out. When stratified according to ethnicity, we detected significant association in Asians (CC *vs* AA: OR = 0.46, 95%CI = 0.27-0.78; CC *vs* AC: OR = 0.41, 95%CI = 0.24-0.71; the dominant model: OR = 2.27, 95%CI = 1.33-3.86; the recessive model: OR = 1.03, 95%CI = 0.86-1.24). Similar results were observed in the population-based subgroups (CC *vs* AA: OR = 0.52, 95%CI = 0.33-0.82; CC *vs* AC: OR = 0.52, 95%CI = 0.32-0.83; the dominant model: OR = 1.92, 95%CI = 1.22-3.02; the recessive model: OR = 0.96, 95%CI = 0.80-1.14).

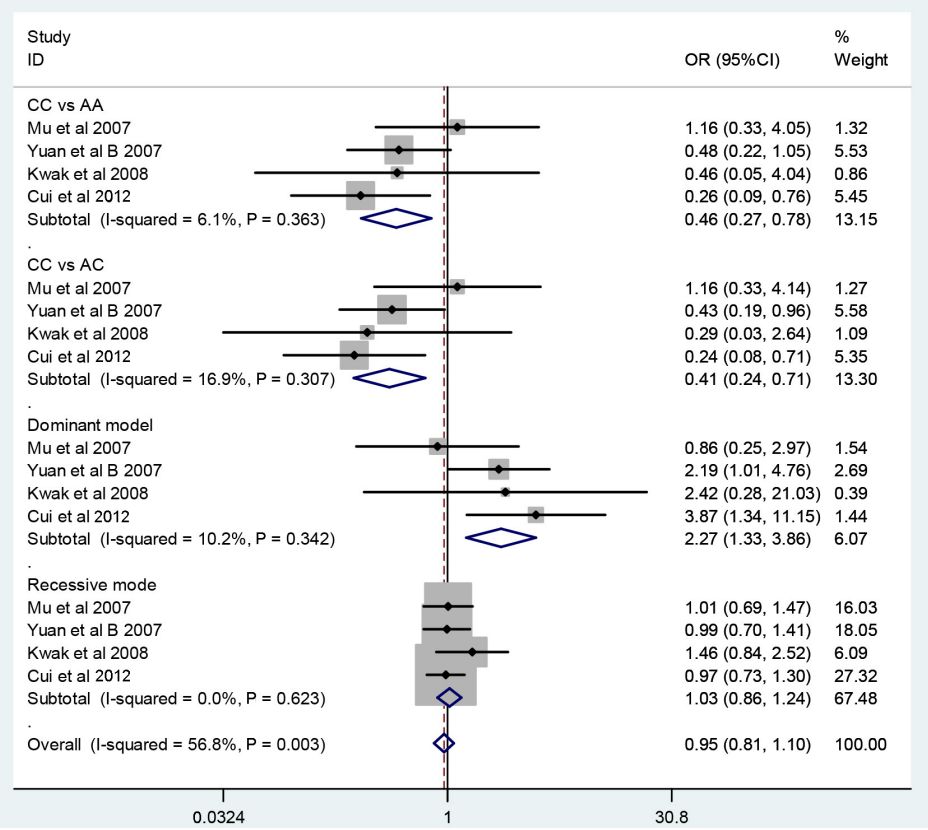

Figure 2. Meta-analysis for the association of MTHFR A1298C polymorphism and HCC in Asians.

Table 3. Summary of different comparative results.

	N	Cases/controls	CC *vs* AA	CC *vs* AC	Dominant model	Recessive mode
Variables			OR (95%CI) P I^2	OR (95%CI) P I^2	OR (95%CI) P I^2	OR (95%CI) P I^2
Total	5	1011/1690	0.52 (0.33-0.81)	0.50 (0.32-0.79)	1.94 (1.24-3.02)	1.00 (0.84-1.18)
			0.43 0.0%	0.25 25.5%	0.35 9.2%	0.64 0.0%
Ethnicity						
Asians	4	893/1481	0.46 (0.27-0.78)	0.41 (0.24-0.71)	2.27 (1.33-3.86)	1.03 (0.86-1.24)
			0.36 6.1%	0.31 16.9%	0.34 10.2%	0.62 0.0%
Caucasians	1	118/209	0.72 (0.31-1.68)	0.87 (0.37-2.07)	1.28 (0.56-2.91)	0.81 (0.51-1.27)
Source of controls						
PB	4	915/1489	0.52 (0.33-0.82)	0.52 (0.32-0.83)	1.92 (1.22-3.02)	0.96 (0.80-1.14)
			0.29 20.7%	0.17 40.8%	0.23 30.8%	0.88 0.0%
HB	1	96 /201	0.46 (0.05-4.04)	0.29 (0.03-2.64)	2.42 (0.28-21.03)	1.46 (0.84-2.52)

N: number; I^2: inconsistency index; OR: odds ratio; CI: confidence interval; PB: population-based study ; HB: hospital-based study.

Publication bias

The Begg test funnel plot was used to assess publication bias in the literature, as shown in Figure 3. There was no evidence of publication bias in our study. The results implied that the publication bias was low in the present meta-analysis (all P > 0.05). Information concerning the Begg's funnel plot is given in Table 3.

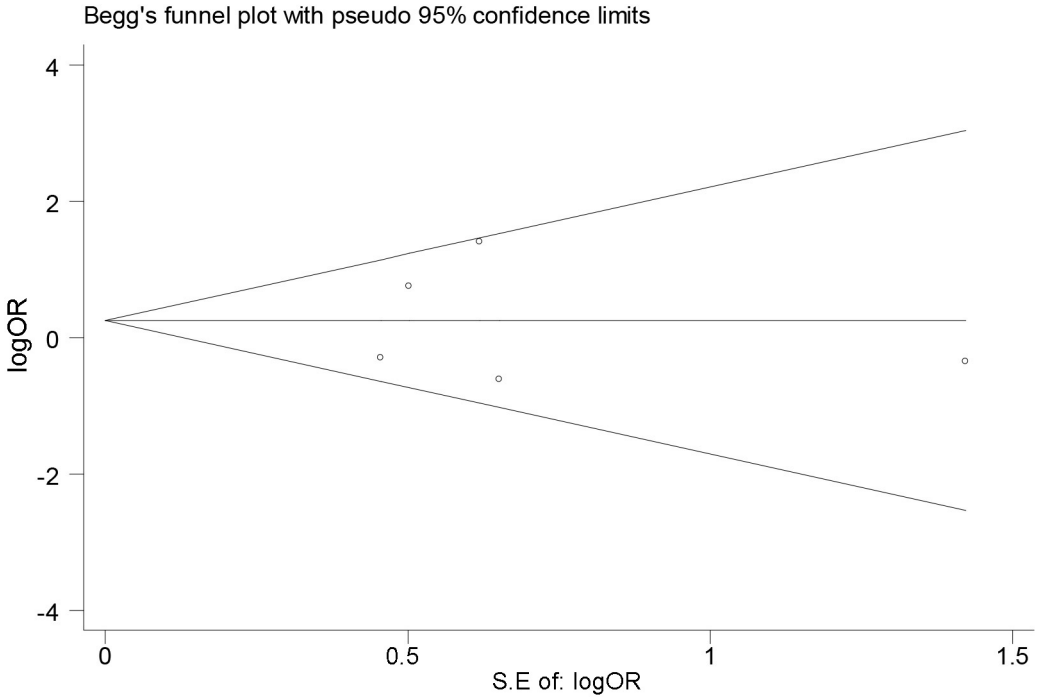

Figure 3. Begg funnel plot test of publication bias for the association between the MTHFR A1298C polymorphism and HCC risk (CC *vs* AA).

DISCUSSION

HCC is a complex disease that involves multistep, multigene, and gene-environment interactions. MTHFR plays a central role in folate metabolism. Gene mutation could affect the function of the *MTHFR* gene and might be associated with HCC risk. In recent years, given the potential role MTHFR plays in the etiology of HCC, more studies have been conducted to identify whether the MTHFR A1298C polymorphism is the genetic determinant of HCC. However, these studies have yielded different or even contradictory results. The most likely reason for these inconsistencies is that the studies are single case-control in design and have small sample sizes. Therefore, we designed this meta-analysis to determine more precisely the association between the MTHFR A1298C polymorphism and HCC risk.

Our meta-analysis quantitatively assessed the association between the MTHFR A1298C polymorphism and HCC risk. Ultimately, five case-control studies from four articles were included and assessed, involving a total of 1011 cases and 1690 healthy controls. The results revealed that the maternal MTHFR A1298C polymorphism was significantly associated with susceptibility to HCC. Considering that the result may be have been affected by ethnicity, we performed a race-related subgroup analysis, and significant association was found in Asians. In the present meta-analysis, only one study of Caucasians was obtained. Further investigations with large sample sizes regarding Caucasians are needed to clarify the possible effects on HCC risk. Hospital-based controls may not always truly reflect the general population, and may thus lead to underestimation of HCC risk. In the subgroup analysis on the source of controls, significantly increased HCC risk was observed in the population-based subgroups, but not in the hospital-based studies. The data of the present meta-analysis indicated that the selection biases hardly affected the results. There was no evidence of publication bias in this meta-analysis of the Arg194Trp gene polymorphism ($P > 0.05$).

The meta-analysis suggested that the MTHFR A1298C polymorphism might be related to the increased risk of HCC in Asians. The mechanism by which the A1298C polymorphism in the *MTHFR* gene relates to HCC risk is still unclear. It has been reported that homozygotes (CC) for A1298C have only 30-60% of the MTHFR enzyme activity (Weisberg et al. 1998). Decreased MTHFR activity may lead to an alteration in the normal intracellular distribution of folate substrates (Bagley and Selhub, 1998), and result in HCC susceptibility. In addition, the potential influence of the MTHFR A1298C polymorphism may take effect via gene-gene interaction. A recent meta-analysis showed that the MTHFR C677T polymorphism is a risk factor for HCC in Asians (Sun et al., 2014); the linkage disequilibrium of C677T and A1298C polymorphisms of the *MTHFR* gene may synergistically increase the risk of HCC.

There were some limitations to our meta-analysis. First, only published studies were included in the meta-analysis, indicating that publication bias may exist even though no obvious publication bias was detected in this analysis. Second, our meta-analysis was based on unadjusted OR estimates because not all published studies presented adjusted ORs, or when they did, the ORs were not adjusted by the same potential confounders, such as age, gender, and exposures. The lack of this information may cause serious bias. Third, the effect of potential gene-gene and gene-environment interactions was not addressed in this meta-analysis. Additionally, meta-analysis is retrospective research that is subject to methodological limitations.

In conclusion, our study indicated that the MTHFR A1298C polymorphism may be associated with HCC in Asians. Studies that take into account gene-gene and gene-environment interactions should be performed to further evaluate this association.

Conflicts of interest

The authors declare no conflict of interest.

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