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Original Article

MTHFR C677T and A1298C polymorphisms and oral cancer risk: an updated meta-analysis

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Abstract: Previous studies have investigated that two functional polymorphisms (C677T, A1298C) of methylenetetrahydrofolate reductase (*MTHFR*) gene may play an vital role in head and neck carcinogenesis, especially oral cancer, however, the association among these two single nucleotide polymorphisms (SNPs) in *MTHFR* gene and the susceptibility of oral cancer (OC) was ambiguous and inconsistent. The objective of our current study was to conduct an update meta-analysis to evaluate the association among *MTHFR* C677T, A1298C SNPs and OC risk. We performed an updated meta-analysis of nine papers involving association between OC and *MTHFR* gene three polymorphisms. We used odds ratios (ORs) with 95% confidence intervals (CIs) to assess the strength of the association. Both in the whole and subgroup analysis, no significant association was found between C677T polymorphism and OS susceptibility. However, for A1298C polymorphism, decreased association was detected in the recessive genetic model (OR = 0.62, 95% CI = 0.41-0.92, *P* = 0.918), moreover, in the subgroup analysis by ethnicity, the similar result was observed (OR = 0.62, 95% CI = 0.41-0.95, *P* = 0.485). To our regret, neither C677T nor A1298C SNP was related to the tumor stage. Results from our current update analysis suggested that A1298C polymorphism in *MTHFR* gene were associated with OC risk.

Keywords: Methylenetetrahydrofolate reductase, oral cancer, polymorphism, tumor stage, risk, meta-analysis

Introduction

The oral cancer (OC) is the eighth most common human cancer worldwide [1]. OC has multifactorial etiology including interactions between genetic background and environmental factors. Methylenetetrahydrofolate reductase (MTHFR) is a central enzyme in the folate pathway that plays crucial and interrelated roles in DNA biosynthesis, methylation, and genomic integrity. MTHFR catalyzes the irreversible conversion of 5,10-methylenetrahydrofolate to 5-methylenetrahydrofolate, which provides one-carbon groups for the methylation of homocysteine to methionine via S-adenosyl methionine (SAM), the universal donor of one-carbon groups. Insufficient DNA methylation or hypomethylation can lead to genomic instability and activation of oncogenes [2-4], so we can presume this gene may influence cancer development, including OC.

MTHFR gene is one of the most studied genes for OC risk. So the basic idea for selecting this particular gene for meta-analysis should have been mentioned as there are myriad of genes that can be used as potential markers. Two common functional single nucleotide polymorphisms (SNPs) in the *MTHFR* gene have been discovered: C677T and A1298C [5, 6]. The C677T SNP is located in the amino-terminal catalytic domain and can lead to a thermolabile enzyme with 35-50% reduced activity [5]. Meanwhile, the A1298C variant is located in the carboxy-terminal regulatory region and lymphocytes from individuals containing 1298CC genotype have been found to have approximately 60% of wild-type in vitro MTHFR activity [7]. Both above SNPs can influence the enzyme activity of MTHFR, which do have functions, however, the concrete mechanism has been unknown.

A number of studies indicated that these two SNPs in *MTHFR* gene were involved in the etiology of OC. Nevertheless, the results from those studies remain conflicting rather than conclusive. Previously, several meta-analyses have been reported, however, the limitation of included samples was all existed in each study. Considering the important role of *MTHFR* gene in oral carcinogenesis, we performed an update analysis on all eligible case-control studies [8-16] or only case studies involving tumor stage to estimate the OC risk associated with two polymorphisms (containing ethnicity, source of control, and tumor stage). To our best of knowledge, this is the most comprehensive meta-analysis conducted to date with respect to the association between *MTHFR* gene two polymorphisms and OC risk.

Methods

Identification of eligible studies and search criterion

A literature search of the PubMed (http://www.ncbi.nlm.nih.gov/), CNKI (http://www.cnki.net/) and Wanfang (http://www.wanfangdata.com.cn/) databases (updated on March 15, 2016) was conducted using combinations of the following keywords 'polymorphism' or 'variant' or 'mutation', 'oral', 'cancer' or 'carcinoma', 'head and neck' and 'MTHFR' or 'methylenetetrahydrofolate reductase'. There was no language restriction. All studies that evaluated the associations between polymorphisms of *MTHFR* gene OC risk were retrieved. Studies that were included in our meta-analysis accord to the following criteria: (1) association between *MTHFR* C677T or A1298C polymorphism and OC risk; (2) case-control design; (3) available frequency of each genotype; (4) some information containing tumor stage. Meanwhile, the following exclusion criteria were also used: (1) no control population; (2) no available genotype frequency; (3) for studies with overlapping or repeating data, the most recent or complete studies with the largest numbers of cases and controls were included and others were excluded.

Data extraction and quality assessment

Information was carefully extracted from all eligible publications independently by two authors (Fangyong Zhu and Jiangang Zou) according to the inclusion criteria listed above. The following data were collected from each study: first author's last name, year of publication, race of origin, sample size (cases/controls), each genotype number in both groups, study design (hospital-based, HB and population-based, PB), Hardy-Weinberg equilibrium (HWE) of controls, genotype method, and age (Mean ± SD) in both case and control groups (**Table 1**). The same two authors (Fangyong Zhu and Jiangang Zou) independently performed the extraction of data and evaluated the study quality based on the Newcastle-Ottawa Scale (NOS) [17]. Total NOS score ranges from 0 to 9. A score ranging 5 to 9 stars is considered to be a generally high methodological quality, whereas a score ranging 0 to 4 is regarded as a relatively poor quality [18].

Statistical analysis

Odds ratios (ORs) with 95% confidence intervals (CIs) were used to measure the strength of the association between *MTHFR* two polymorphisms and OC based on the genotype frequencies in cases and controls. In our analysis, we recognized 677T or 1298C as 'M', and C677 or A1298 as 'W'. We analyzed this relationship between C677T or A1298C and OC risk using five different genetic models: allelic contrast (M vs. W), heterozygote comparison (MW vs. WW), homozygote comparison (MM vs. WW), dominant genetic model (MM+MW vs. WW) and recessive genetic model (MM vs. MW+WW), respectively. Different ethnic descents were categorized as Caucasian and Asian. Furthermore, tumor stage was also performed, OC were classified into early (I-II or well and moderately differentiated) and advanced (III-IV or poorly differentiated) stages [19, 20].

Heterogeneity assumption was evaluated with a chi-square-based *Q*-test and considered statistically significant at $P < 0.10$ [21]. When *P* for heterogeneity test (P_h) > 0.10, the pooled OR of each study was calculated by using the fixed-effects model (the Mantel-Haenszel method, which weights the studies by the inverse of the variance of estimates); otherwise, the random-effects model (the DerSimonian and Laird method) was used [22, 23]. The statistical significance of the summary OR was determined with the *Z*-test. The funnel plot asymmetry and publication were assessed with Begg's test, $P < 0.05$ was considered statistically significant [24]. The departure of frequencies of *MTHFR*

Table 1. Study characteristics from published studies on the relationship between two polymorphisms in MTHFR gene and oral cancer

First author	Year	Origin	Ethnicity	Design	Case	Control	Case			Control				Method	NOS score	Age (Mean ± SD)	
							TT	TC	CC	TT	TC	CC	HWE			Case	Control
MTHFR C677T																	
Tsai	2011	China/Taiwan	Asian	HB	620	620	43	186	391	62	236	322	0.057	PCR-RFLP	6	65.5 ± 9.7	63.5 ± 8.5
Solomon	2008	India	Asian	HB	126	100	23	55	48	10	42	48	0.855	PCR-RFLP	6	54.5 ± 8.6	55.4 ± 10
Sailasree	2011	India	Asian	HB	101	138	1	8	92	1	29	108	0.527	PCR-RFLP	6	NA	NA
Vairaktaris	2006	Germany	Caucasian	PB	110	120	6	76	28	10	65	45	0.044	PCR-RFLP	6	52.1 ± 7.2	51.5 ± 5.5
Supic	2011	Serbia	Caucasian	HB	96	162	14	32	50	16	66	80	0.661	PCR-RFLP	6	NA	NA
Vylliotis	2013	Greece	Caucasian	PB	110	120	6	28	76	10	45	65	0.580	PCR-RFLP	6	58.5 ± 10.1	54.7 ± 11.9
Weinstein	2002	Puerto Rico	Caucasian	PB	135	146	15	53	67	15	62	69	0.846	PCR-RFLP	5	63.2 ± NA	61.0 ± NA
Miri-Moghaddam	2015	Iran	Caucasian	HB	57	62	2	21	34	1	14	47	0.971	PCR-ARMS	7	55 ± 17	48 ± 12
Barbosa	2015	Brazil	Mixed	HB	101	102	6	45	50	11	41	50	0.555	PCR-RFLP	7	NA	NA
MTHFR A1298C																	
Tsai	2011	China-Taiwan	Asian	HB	620	620	21	192	407	29	198	393	0.528	PCR-RFLP	6	65.5 ± 9.7	63.5 ± 8.5
Sailasree	2011	India	Asian	HB	130	139	19	74	37	34	59	46	0.088	PCR-RFLP	6	NA	NA
Miri-Moghaddam	2015	Iran	Caucasian	HB	57	62	1	26	30	2	26	34	0.259	PCR-ARMS	7	55 ± 17	48 ± 12
Barbosa	2015	Brazil	Mixed	HB	99	102	3	36	60	5	44	53	0.275	PCR-RFLP	7	NA	NA

HWE: Hardy-Weinberg equilibrium of controls; NOS: Newcastle-Ottawa Scale; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; PCR-ARMS: polymerase chain reaction-amplification refractory mutation system. NOS: Newcastle-Ottawa Scale; NA: not available

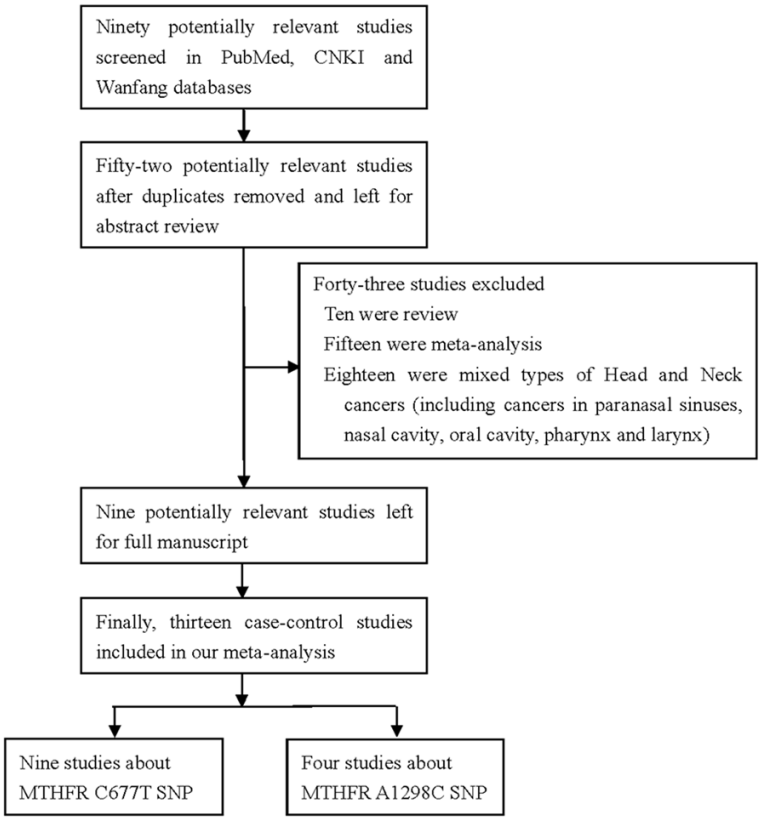

Figure 1. Flowchart illustrating the search strategy for *MTHFR* two polymorphisms and the risk of OC.

polymorphisms from expectation under HWE was assessed by χ^2 test in controls using the Pearson chi-square test, $P < 0.05$ was also considered significant. All statistical tests for this meta-analysis were performed with Stata software (version 10.0; StataCorp LP, College Station, TX).

Results

Studies characteristics

Total 90 potentially relevant publications were searched in PubMed, CNKI and Wanfang databases. 52 potentially papers were left for abstract review after irrelevant titles removed. 43 were excluded: review (10), meta-analysis (15), mixed types of head and neck cancers including paranasal sinuses, nasal cavity, oral cavity and pharynx and larynx cancers (18). Finally, we identified 9 different articles (13 case-control studies: 9 studies about C677T SNP [8-16] and 4 studies about A1298C SNP) [8, 9, 14, 15] (**Table 1**; **Figure 1**) to evaluate the association of *MTHFR* gene two polymorphisms with risk for OC. The included OC cases were oral squamous cell carcinoma (OSCC) at most, in addition, the control group were cancer-free healthy subjects or individuals without history of malignant disease. Three studies [8, 9, 11] included information tumor stage. Study characteristics were seen in **Table 1**. The distribution of genotypes in the controls was consistent with HWE in all studies. For the C677T polymorphism, containing 9 case-control studies (1,456 cases and 1,570 controls), there had 5 studies of Caucasian, 3 of Asian. For the A1298C polymorphism, containing 4 case-control studies (906 cases and 923 controls). Genotyping for SNP of *MTHFR* gene polymorphisms was conducted using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and PCR-amplification refractory mutation system (ARMS).

Quantitative synthesis

Overall, there had not obvious significantly relationships between C677T polymorphism and OC risk in all available genotype models (C-allele vs. T-allele: OR = 0.93, 95% CI = 0.73-1.20, $P_{heterogeneity}$ = 0.000; CC vs. TT: OR = 0.84, 95% CI = 0.64-1.09, $P_{heterogeneity}$ = 0.109; CT vs. TT: OR = 0.90, 95% CI = 0.65-1.25, $P_{heterogeneity}$ = 0.001; CC+CT vs. TT: OR = 0.92, 95% CI = 0.67-1.27, $P_{heterogeneity}$ = 0.000; CC vs. CT+TT: OR = 0.89, 95% CI = 0.69-1.16, $P_{heterogeneity}$ = 0.220). Moreover, in the stratified analysis by race and source of control subgroups, the negative results were also found (**Table 2**). On the other hand, for A1298C SNP, a decreased association was found in both total (OR = 0.62, 95% CI = 0.41-0.92, $P_{heterogeneity}$ = 0.918, **Figure 2**) and Asian (OR = 0.62, 95% CI = 0.41-0.95, $P_{heterogeneity}$ = 0.485, **Figure 2**) subgroup in the recessive genetic model (**Table 2**).

Table 2. Total and stratified analysis of two polymorphisms in MTHFR gene on oral cancer

Variables	N[a]	Case/Control	M-allele vs. W-allele		MW vs. WW		MM vs. MW+WW		MM vs. WW		MM+MW vs. WW	
			OR (95% CI)	P_h[b]	OR (97% CI)	P_h[b]	OR (99% CI)	P_h[b]	OR (95% CI)	P_h[b]	OR (95% CI)	P_h[b]
MTHFR C677T												
Total	9	1456/1570	0.93 (0.73-1.20)	0.000	0.90 (0.65-1.25)	0.001	0.89 (0.69-1.16)	0.220	0.84 (0.64-1.09)	0.109	0.92 (0.67-1.27)	0.000
Ethnicity												
Caucasian	5	508/610	1.02 (0.76-1.38)	0.049	1.04 (0.64-1.68)	0.010	1.03 (0.68-1.58)	0.530	1.02 (0.66-1.59)	0.538	1.06 (0.67-1.66)	0.011
Asian	3	847/858	0.79 (0.42-1.47)	0.001	0.69 (0.37-1.27)	0.015	1.11 (0.45-2.74)	0.051	1.09 (0.34-3.46)	0.014	0.72 (0.37-1.43)	0.003
Source of control												
HB	6	1101/1184	0.95 (0.67-1.36)	0.000	0.88 (0.59-1.31)	0.005	1.06 (0.62-1.81)	0.088	1.04 (0.56-1.96)	0.036	0.91 (0.60-1.39)	0.001
PB	3	355/386	0.90 (0.62-1.33)	0.057	0.96 (0.48-1.90)	0.009	0.82 (0.49-1.39)	0.604	0.84 (0.48-1.45)	0.566	0.94 (0.49-1.79)	0.011
MTHFR A1298C												
Total	4	906/923	0.89 (0.76-1.03)	0.861	0.98 (0.81-1.20)	0.252	0.62 (0.41-0.92)	0.918	0.68 (0.44-1.03)	0.986	0.93 (0.77-1.13)	0.494
Asian	2	750/759	0.90 (0.75-1.06)	0.975	1.13 (0.70-1.84)	0.097	0.62 (0.41-0.95)	0.485	0.70 (0.45-1.09)	0.989	0.96 (0.77-1.18)	0.276

[a]Number of comparisons, [b]*P* value of *Q*-test for heterogeneity test.

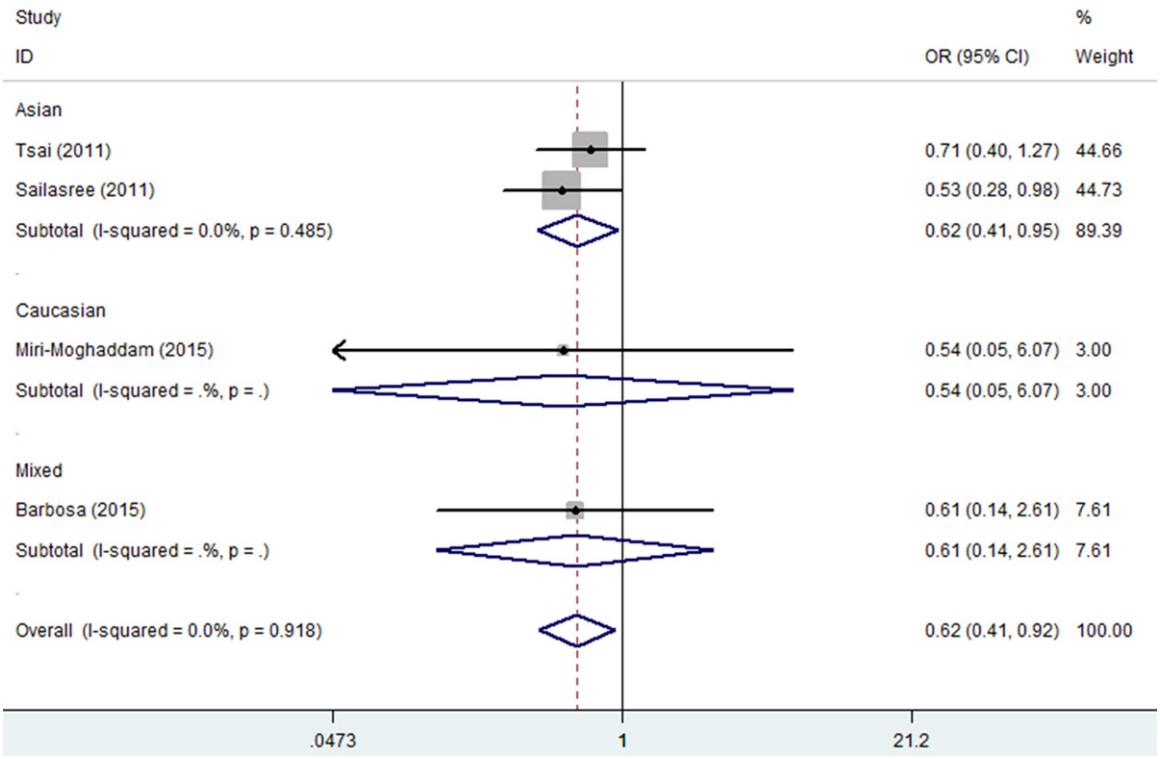

Figure 2. Forest plot of OC risk associated with the *MTHFR* A1298C polymorphism (recessive genetic model) in total and ethnicity subgroup. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the weight (inverse of the variance). The diamond represents the summary OR and 95% CI.

Table 3. Genotype distribution of two polymorphisms in oral cancer's tumor stage

Variables	Advanced-stage			Early-stage		
	TT	TC	CC	TT	TC	CC
MTHFR C677T						
Vylliotis (2013)	2	13	34	4	15	42
Miri-Moghaddam (2015)	1	13	19	1	8	15
Barbosa (2015)	4	34	38	2	11	12
	CC	CA	AA	CC	CA	AA
MTHFR A1298C						
Barbosa (2015)	4	26	46	1	10	14
Miri-Moghaddam (2015)	0	16	17	1	10	13

Moreover, we tried to analysis whether these two MTHFR SNPs may be associated with the oral cancer's stage, which had more significant means and may be a biomarker in the oral cancer's follow-up. To our regret, no meaningful result was detected in both SNPs (**Table 3**).

Sensitivity analysis and publication bias diagnosis

We deleted each study involved in our meta-analysis to reflect the influence of the individual data-set to the pooled OR and the corresponding pooled OR was not materially altered indicating that our results were statistically robust. The Begg's funnel plot was performed to access the publication bias. The shape of the funnel plot revealed no obvious asymmetry and the absence of publication bias was not existed in each *MTHFR* polymorphism (TT vs. TC+CC: z = 0.42, P = 0.677 for C677T SNP; CC vs. CA+AA: z = 0.34, P = 0.734 for A1298C SNP, **Figures 3**, **4**).

Discussion

Folic acid is essential for normal DNA synthesis and normal cellular methylation reactions. The enzyme about MTHFR can catalyze the synthesis of 5-methylenetetrahydrofolate, the methyl donor for the B12-dependent remethylation of homocysteine to methionine, which is the precursor for SAM, the major cellular methyl donor for DNA, RNA, proteins and phospholipids methylation [3, 4]. Taking into consideration that abnormal DNA synthesis and cellular methylation reactions promotes the development of

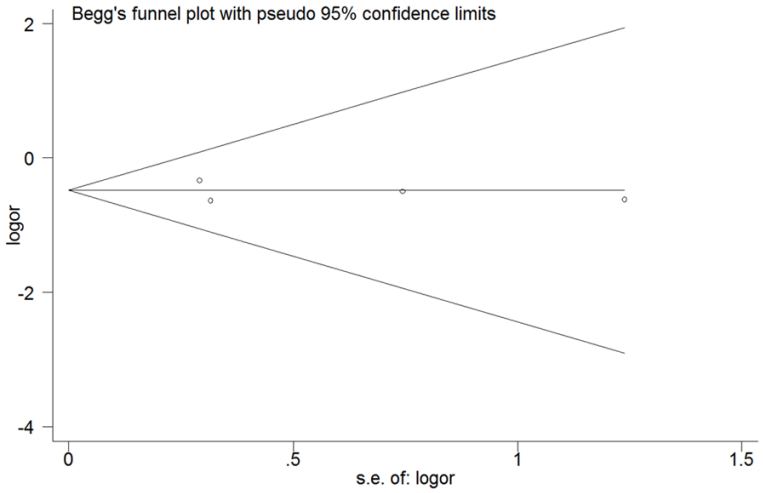

Figure 3. Begg's funnel plot for publication bias test (TT vs. TC+CC in *MTHFR* C677T SNP). Each point represents a separate study for the indicated association. Log [OR], natural logarithm of OR. Horizontal line, mean effect size.

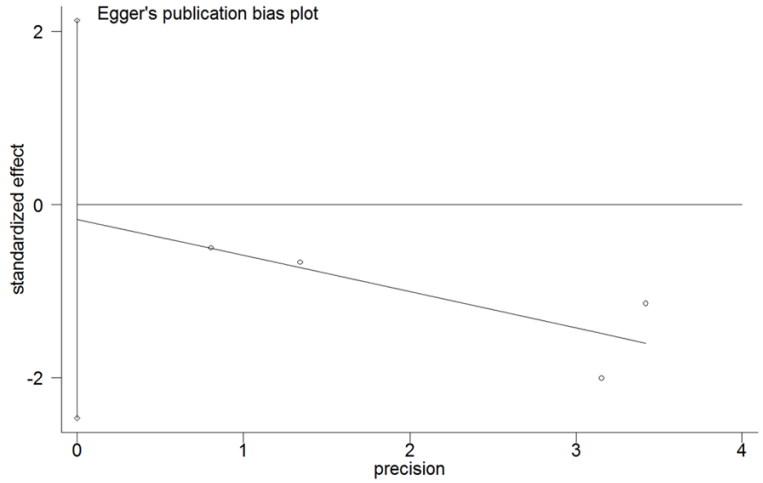

Figure 4. Begg's funnel plot for publication bias test (CC vs. CA+AA in *MTHFR* A1298C SNP). Each point represents a separate study for the indicated association. Log [OR], natural logarithm of OR. Horizontal line, mean effect size.

cancer, including OC, a number of polymorphisms of genes encoding for folate pathway have been studied and a correlation with OC risk has been found. Hence, the above pathways might be affected by the *MTHFR* C677T or A1298C functional polymorphism which could both reduce enzyme activity of MTHFR, so it makes sense to research the association between these two *MTHFR* polymorphisms and cancer risk and the might mechanisms, including OC.

The overall goal of pooled analysis is to combine the results of previous studies to arrive at summary conclusions about a body of research. It is most useful in summarizing prior research when individual studies are small, and when they are individually too small to yield a valid conclusion. To provide further insights into this debated subject, an update meta-analysis is needed to achieve a more reliable and comprehensive conclusion on both variants. So far, some meta-analysis have been reported and suggested that these two *MTHFR* polymorphisms may associate with the risk of breast cancer, prostate cancer, endometrial cancer, colorectal cancer [25-28], and so on. To the best of our knowledge, this is the updated analysis to explore the association between *MTHFR* gene polymorphisms and OC risk, involving about 1,456 cases and 1,570 controls of C677T polymorphism and 906 cases and 923 controls of A1298C polymorphism [8-16]. We found that A1298C polymorphism was associated with significant decrease in OC risk, suggesting C-allele was a protective factor in OC individuals. We want to find a new blood serum marker through analyzing relationship between *MTHFR* two SNPs and tumor stage of OC, so can detect possible high-risk patients, meanwhile intervene or treat in advance. To our regret, no such association was observed in our meta-analysis.

We have put considerable efforts and resources into testing possible association between *MTHFR* two polymorphisms and OC risk, but there are still some limitations inherited from the published studies. First of all, sample size varied widely in different studies (range of no. of cases/controls 57 to 620), which maybe increase the publication bias in our analysis, further study will consider this problem. Second, not enough data about two SNPs and

OC tumor stage were available, further studies are needed to deal with these problems and pay more attention to gene-gene and gene-environment (such as smoking stage, drinking stage, sex and family history) interactions, because cancer is a multi-factorial disease that results from complex interactions between many genetic and environmental factors, which means that there do not be a single gene or single environmental factor that has large effects on cancer susceptibility [29]. Third, in our meta, quality score system-Cochrane quality criterion was not applied.

Our present update-analysis found novel evidence that *MTHFR* A1298C polymorphism played protective effects on OC risk. Further prospective studies with larger numbers of worldwide individuals are expected to examine associations between these two polymorphisms in *MTHFR* and OC risk and its prognostic.

Disclosure of conflict of interest

None.

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