RESEARCH ARTICLE

5,10-Methylenetetrahydrofolate Reductase Polymorphisms and Colon Cancer Risk: a Meta-analysis

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Abstract

Previous studies investigating the association between 5,10-methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and colon cancer risk have generated conflicting results. The aim of our meta-analysis was to clarify the precise association. A systematic literature search was conducted to identify all relevant studies. Pooled odds ratio (ORs) with 95% confidence interval (CI) were used to estimate the strength of the association. In this meta-analysis, a total of 13 articles, involving 5,386 cases and 8,017 controls met the inclusion criteria. Overall, a significant association was found between colon cancer risk and the MTHFR C667 polymorphism (TT *vs* CC+CT: OR=0.79; 95%CI=0.65-0.96; *p*=0.017). Stratification by ethnicity revealed that MTHFRC667 was associated with colon cancer risk in the non-Asian group (TT *vs* CC+CT:OR=0.77, 95%CI=0.68-0.89, *p*=0.000; TT *vs* CC: OR=0.84, 95%CI=0.73-0.97, *p*=0.016). Stratification by source of control indicated that MTHFR C667 also correlated with colon cancer risk in the population-based subgroup (TT *vs* CC: OR=0.85, 95%CI=0.74-0.97, *p*=0.017; TT *vs* CC+CT: OR=0.78, 95%CI=0.68-0.89, *p*=0.000) and hospital-based subgroup (TT *vs* CC+CT: OR=0.65, 95%CI=0.49-0.86, *p*=0.003). However, risk was significantly increased for MTHFR A1298C polymorphisms and colon cancer risk in hospital-based studies (C *vs* A: OR=1.52, 95%CI=1.26-1.83, *p*=0.000; CC+AC *vs* AA: OR=1.93, 95%CI=1.47-2.49, *p*=0.000) but reduced in population-based studies (CC *vs* AA: OR=0.83, 95%CI=0.70-0.99, *p*=0.042). In conclusion, the results of our meta-analysis suggest that the MTHFR C667 polymorphism is associated with reduced colon cancer risk, especially for non-Asian populations.

Keywords: 5, 10-methylenetetrahydrofolate reductase - polymorphisms - colon cancer - meta-analysis - non-Asians

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Introduction

Colon cancer is a complex disease involving multiple genetic and environmental factors. It is the fourth leading cause of cancer-related mortality among males and the third most deadly cancer among females. Furthermore, the incidence rates vary approximately 20-fold around the world and the highest rates are seen largely in developing countries (Potter et al., 1993). Epidemiological studies have indicated that diets with a high intake of vegetables, fruit and dietary fiber are associated with an decreased colon cancer risk (Boutron-Ruault et al., 2001; Giovannucci, 2001). It is well known that vegetables, particularly green, leafy vegetables, are a major source of folate.

5, 10-methylenetetrahydrofolate reductase (MTHFR), a central enzyme in folate metabolism, catalyzes the reduction of 5, 10-methyl- enetetrahydrofolate to 5-methyltetrahydrofolate (Choi and Mason, 2000). 5-methyltetrahydrofolate is the main circulatory form of folate in the body and is used as a methyl donor for converts homocysteine to methionine, the precursor of S-adenosylmethionine (SAM). Deficient in methyl groups, lowers the concentration of SAM, possibly reducing DNA methylation and decreasing the synthesis of thymidine from uracil, resulting in misincorporation of uracil in place of thymidine, leading to DNA strand breaks (Blount and Ames, 1995). In conclusion, MTHFR plays a pivotal role in folate metabolism (Stern et al., 2000), and is an important factor in DNA methylation, synthesis, and repair (Ueland et al., 2001).

Two common single nucleotides polymorphisms, the C677T (1p36.22, rs1801133) and A1298C (1p36.22, rs1801131) in the MTHFR gene, have been identified (Chen et al., 1996; Ma et al., 1997; Le Marchand et al., 2002). The MTHFR 677 C>T transition in exon 4 (C3T, ala to val) and MTHFR 1298 A>C transversion in exon 7 (A3C, gluto ala) are associated with decreased activity of the MTHFR enzyme (Frosst et al., 1995). Increasing evidence from epidemiological studies indicated that the MTHFR C677T and A1298C polymorphisms can exert effect on the development of malignant tumors, such as clear cell renal cell carcinoma, liver cancer, and gastric cancer (Safarinejad et al., 2012; Liang et al., 2014; Lv et

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al., 2014). Nonetheless, the effect varies across different cancers, and the reason for this discrepancy needs further elucidation.

Previous studies investigated the association between MTHFR C677T polymorphism and colon carcinoma risk, but the impact of MTHFR polymorphism on colon carcinoma was still conflicting due to inconsistent findings in individual studies (Slattery et al., 1999; Keku et al., 2002; Toffoli et al., 2003; Kim et al., 2004; Jiang et al., 2005; Wang et al., 2006; Curtin et al., 2007; Cao et al., 2008; Komlosi et al., 2010; Promthet et al., 2010; Jokic et al., 2011; Kim et al., 2012; Rai et al., 2014). Therefore, the current meta-analysis aimed to quantify the strength of the association between MTHFR polymorphisms and colon cancer risk by pooling data from all available case-control studies.

Materials and Methods

Publication search

A systematic literature search in PubMed, Elsevier Science Direct, the China National Knowledge Infrastructure database (CNKI), and the Chinese Biomedical database (CBM) was performed to identify articles. References in the studies were reviewed to find additional studies regarding the association between MTHFR polymorphisms and colon cancer risk. The following keywords were used for searching: 'methylenetetrahydrofolate reductase' or 'MTHFR', 'polymorphism' or 'mutation' or 'variant', 'colon', and 'cancer' or 'carcinoma'. The search was conducted without restriction on language. The last search was updated on April 1, 2014.

Inclusion and exclusion criteria

Studies included in our meta-analysis had to meet the following inclusion criteria: (1) evaluation of MTHFR gene polymorphisms (C667T, A1298C) and colon cancer risk, (2) using a case-control design, (3) genotype distributions in both cases and controls should be available for estimating an odds ratio (OR) with 95% confidence interval (CI), (4) genotype distribution of control population had to be consistent with Hardy-Weinberg equilibrium (HWE), and (5) if there were various publications from the same population, only the most recent or complete study was included. Studies were excluded if one of the following existed: (1) the studies contained overlapping data. (2) the genotype or allele frequencies were not reported. (3) the studies design was not case-control. (4) the association between MTHFR polymorphisms and colon cancer was not investigated. (5) the studies investigating progression, severity, phenotype modification, response to treatment, or survival.

Data extraction

Data were collected carefully and independently by two independent investigators (Xin-yu Fang and Qian Huang). The characteristics of the selected articles are shown in Table 1, including first author's name, publication year, study populations, ethnicity, source of controls, number of cases and controls, findings about the polymorphisms investigated in these studies, and HWE (*P* value).

Statistical analysis

Allele frequencies at the MTHFR gene polymorphisms from the individual study were determined by the counting method. The overall odds ratio (OR) with the corresponding 95% confidence interval (95%CI) was used to assess the strength of the association between the gene polymorphisms and colon cancer susceptibility. HWE was tested using the χ^2 test (significant at the 0.05 level). The χ^2 test-based Q statistic was used to examine the between-study heterogeneity (Higgins and Thompson, 2002). The I^2 statistic measures the degree of inconsistency in the studies by computing what percentage of the total variation across studies was due to heterogeneity rather than by chance. I^2 values of 25, 50, and 75 % were used as evidence of low, moderate, and high heterogeneities, respectively (I^2 =0% to 25%, no heterogeneity; I^2 =25% to 50%, moderate heterogeneity; I^2 =50% to 75%, large heterogeneity; and I^2 =75% to 100%, extreme heterogeneity). If the *P* value of the heterogeneity Q statistic was less than 0.05, the random effects model was selected; Otherwise, the fixed effects model was used. The study populations comprised Koreans, Indians, Hungarians, Chinese, Italians, Thais, Croats and Americans. In our study, ethnicities were classified as Asian and Non-Asian subgroup. Publication bias was estimated using Begg's funnel plot and Egger's test. An asymmetric plot suggested possible publication bias and the degree of asymmetry was assessed via *P* value. If the *P* value was less than 0.05, statistically significant publication bias might exist (Egger et al., 1997). All *P* values were two-sided and *P* value below 0.05 was considered statistically significant. All the statistical analyses for the meta-analysis were performed with STATA statistical software (version12.0 STATA Corp, College Station, TX).

Results

Flow of eligible studies

A total of 275 publications were relevant to the search terms. Among these literatures, 230 studies was excluded owing to not about MTHFR gene polymorphism, 17 were not about colon cancer, and 1 was review. The remaining 27 studies were selected for detailed evaluation. Among them, 1 study were excluded for sharing the same case and control, 10 studies were excluded because it was not a case-control design, and 3 were exclude owing to the absence of genotype frequencies. Finally, 13 articles involving 5 386 cases and 8 017 controls concerning MTHFR polymorphisms and colon cancer risk were included in the present meta-analysis (Figure 1).

Study characteristics

A total of 275 studies were identified during the literature search and 13 articles involving 5 386 cases and 8 017 controls met the inclusion criteria and were finally included in our meta-analysis. One study, conducted by Temitope Keku et al. (2002), consisted of two individual case-control studies and was handled as two populations.

The number of cases varied from 59 to 1, 467, while the numbers of controls varied from 130 to 1, 972. The main characteristics of each study included in this meta-analysis are shown in Table 1.

Main results and subgroup analyses

Table 2 listed the main results of this meta-analysis. 13 studies determined the relationship between the C677T polymorphism and colon cancer risk (Slattery et al., 1999; Keku et al., 2002; Toffoli et al., 2003; Kim et al., 2004; Jiang et al., 2005; Wang et al., 2006; Curtin et al., 2007; Cao et al., 2008; Komlosi et al., 2010; Promthet et al., 2010; Jokic et al., 2011; Kim et al., 2012; Rai et al., 2014). The total sample size for patients with colon cancer and healthy controls was 5 386 and 8 017, respectively. The overall result revealed that MTHFR C677T polymorphism was associated with a reduced colon cancer risk under the recessive model (TT *vs* CC+CT: OR=0.79; 95%CI=0.65-0.96; *p*=0.017) (Figure 2). After stratified by ethnicity, a significant association between MTHFR C677T polymorphism and colon cancer risk was observed in the Non-Asian population (TT *vs* CC: OR=0.84, 95%CI=0.73-0.97, *p*=0.016; TT *vs* CC+CT: OR= 0.77, 95%CI=0.68-0.89, *p*=0.000) (Figure 3A, 3B).

Eight studies containing 2 549 cases and 6 305 controls examined the association of MTHFR A1298C and colon cancer risk. However, no significant association was found between the MTHFR A1298C polymorphism and colon cancer. Notably, the same results were observed after stratifying by ethnicity.

Furthermore, when stratifying by control source, not only in the population-based group we could see the relationship between MTHFR C677T polymorphisms and colon cancer risk (TT *vs* CC: OR=0.85, 95%CI=0.74-

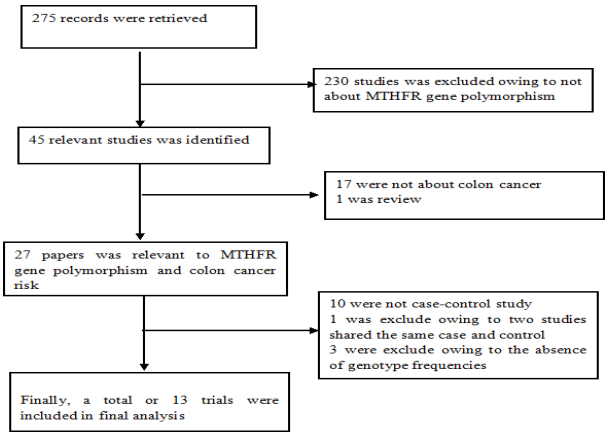

Figure 1. Process of Selecting Studies

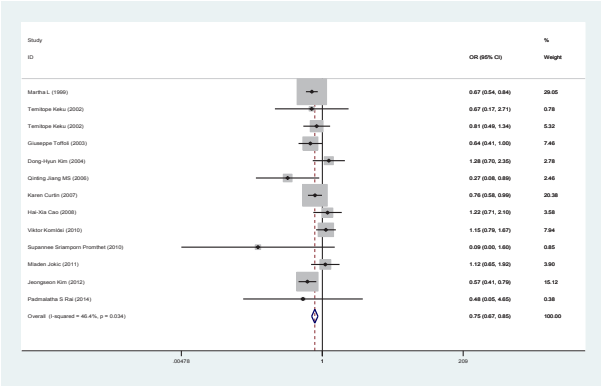

Figure 2. Odds Ratios and 95% Confidence Intervals for Individual Studies and Pooled Data for the Association between the TT *versus* CC+CT of the MTHFR C677T Polymorphism and Colon Cancer

Table 1. Characteristics of Individual Studies Included in the Meta-Analysis

First author	Year	Population	Ethnicity	Source of controls	Sample size		Polymorphism	*P* value of HWE
					Cases	Controls		
Temitope Keku	2002	USA	Non-Asian	PBS	311	544	C677T	0.214
							A1298C	0.016
			Non-Asian	PBS	244	331	C677T	0.681
							A1298C	0.000
Mladen Jokic	2011	Croatia	Non-Asian	PBS	300	300	C677T	0.823
							A1298C	0.734
Jingwen Wang	2006	India	Asian	PBS	59	291	C677T	0.261
							A1298C	0.505
Jeongseon Kim	2012	Korea	Asian	HBS	787	656	C677T	0.003
Qinting Jiang MS	2006	Chinese	Asian	PBS	53	343	C677T	0.032
							A1298C	0.137
Karen Curtin	2007	USA	Non-Asian	PBS	916	1972	C677T	0.373
							A1298C	0.119
Padmalatha S Rai	2014	India	Asian	HBS	155	294	C677T	0.250
							A1298C	0.001
Dong-Hyun Kim	2004	Korea	Asian	HBS	111	225	C677T	0.773
Viktor Komlósi	2010	Hungary	Non-Asian	PBS	472	461	C677T	0.061
Promthet	2010	Thailand	Asian	HBS	130	130	C677T	0.243
							A1298C	0.002
Hai-Xia Cao	2008	China	Asian	PBS	105	370	C677T	0.824
							A1298C	0.700
Giuseppe Toffoli	2003	Italy	Non-Asian	PBS	276	279	C677T	0.827
							A1298C	0.735
Martha L	1999	USA	Non-Asian	PBS	1467	1821	C677T	0.341

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

Table 2. Meta-Analysis of the MTHFR Gene Polymorphisms in Colon Cancer

Polymorphic nucleotide	Population	No. of studies	Sample size Cases	Controls	Test of accociation OR (95%CI)	Z	*P*	Test of heterogencity Model	X^2	*P*	I^2(%)	Egger's test *P* value
C677T												
T *vs* C	Overall	13	5386	8017	0.98(0.89,1.07)	0.51	0.611	R	27.5	0.011	52.7	0.545
	Asian	7	1400	2309	1.01(0.77,1.31)	0.05	0.957	R	21.43	0.002	72	0.727
	Non-Asian	6	3986	5708	0.95(0.89,1.01)	1.61	0.107	F	5.96	0.428	0	0.659
TT *vs* CC	Overall	13	5386	8017	0.86(0.70,1.06)	1.44	0.150	R	22.85	0.029	47.5	0.829
	Asian	7	1400	2309	0.80(0.41,1.57)	0.65	0.519	R	16.25	0.006	69.2	0.790
	Non-Asian	6	3986	5708	0.84(0.73,0.97)	2.41	0.016	F	6.64	0.356	9.6	0.821
TT *vs* CC+CT	Overall	13	5386	8017	0.79(0.65,0.96)	2.39	0.017	R	22.37	0.034	46.4	0.874
	Asian	7	1400	2309	0.71(0.41,1.23)	1.23	0.218	R	13.95	0.016	64.1	0.649
	Non-Asian	6	3986	5708	0.77(0.68,0.89)	3.68	0.000	F	8.37	0.212	28.3	0.469
TT+CT *vs* CC	Overall	13	5386	8017	1.01(0.94,1.09)	0.22	0.826	F	21.18	0.069	38.6	0.287
	Asian	7	1400	2309	1.16(0.86,1.57)	0.99	0.322	R	15.61	0.016	61.6	0.792
	Non-Asian	6	3986	5708	0.98(0.90,1.00)	0.49	0.628	F	3.35	0.764	0	0.697
A1298C												
A *vs* C	Overall	9	2549	6305	1.01(0.85,1.20)	0.11	0.911	R	37.07	0.000	75.7	0.851
	Asian	5	1263	2879	0.99(0.66,1.47)	0.06	0.950	R	23.28	0.000	82.8	0.073
	Non-Asian	4	1286	3426	1.06(0.97,1.16)	1.36	0.175	F	6.11	0.191	34.5	0.909
AA *vs* CC	Overall	9	2549	6305	1.11(0.79,1.57)	0.59	0.555	R	25.07	0.003	64.1	0.587
	Asian	5	1263	2879	1.39(0.39,4.93)	0.51	0.612	R	18.34	0.001	78.2	0.213
	Non-Asian	4	1286	3426	1.13(0.95,1.36)	1.37	0.171	F	4.78	0.311	16.3	0.784
CC *vs* AA+AC	Overall	9	2549	6305	0.89(0.68,1.16)	0.88	0.381	R	17.05	0.048	47.2	0.367
	Asian	5	1263	2879	0.71(0.26,1.85)	0.7	0.486	R	10.78	0.029	62.9	0.152
	Non-Asian	4	1286	3426	0.89(0.75,1.05)	1.36	0.174	F	5.74	0.220	30.3	0.621
CC+AC *vs* AA	Overall	9	2549	6305	1.07(0.87,1.31)	0.62	0.536	R	31.14	0.000	71.1	0.959
	Asian	5	1263	2879	1.19(0.77,1.86)	0.79	0.430	R	18.08	0.001	77.9	0.070
	Non-Asian	4	1286	3426	0.98(0.88,1.10)	0.36	0.721	F	4.13	0.389	3.2	0.583

F fixed-effects model, R random-effects model

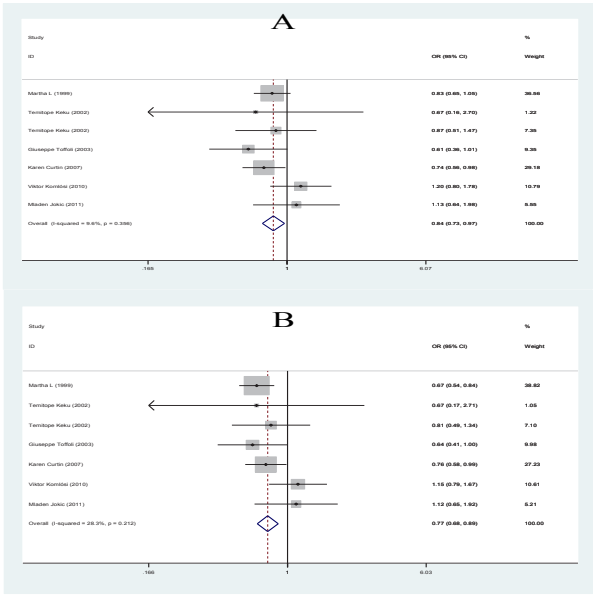

Figure 3. A. Odds Ratios and 95% Confidence Intervals for the Association between the CC versus TT of the MTHFR C677T Polymorphism and Colon Cancer in the Non-Asian Subgroup. B. Odds Ratios and 95% Confidence Intervals for the Association between the TT versus CC+CT of the MTHFR C677T Polymorphism and Colon Cancer in the Non-Asian Subgroup

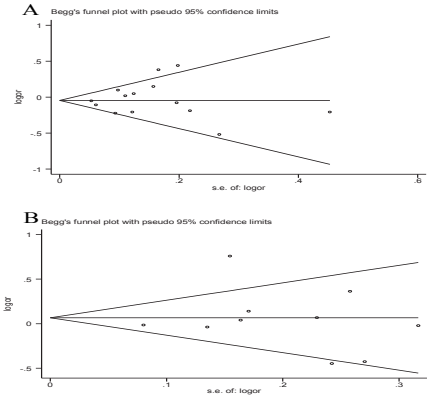

Figure 4. A Begg's Test Assessment of Publication Bias for the C677T Polymorphism and Colon Cancer (T verus C); B Begg's Test Assessment of Publication Bias for the A1298C Polymorphism and Colon Cancer (TT verus CC)

0.97, *p*=0.017; TT *vs* CC+CT: OR=0.78, 95%CI=0.68-0.89, *p*=0.000) but also in hospital-based group (TT *vs* CC+CT: OR=0.65, 95%CI=0.49-0.86, *p*=0.003). (Table 3). However, statistically significantly increased risks were found between MTHFR A1298C polymorphism and colon cancer risk in hospital-based studies (C *vs* A: OR=1.52,

Table 3. Association of MTHFR C677T and A1298C Polymorphisms with Colon Cancer Risk Which Stratified by Source of Controls (HBS and PBS)

Polymorphisms	Test of accociation (PBS) OR(95% CI)	*P* value	Test of accociation (HBS) OR(95% CI)	*P* value
C677T				
T *vs* C	0.95(0.90,1.01)	0.124	1.03(0.68,1.57)	0.882
TT *vs* CC	0.85(0.74,0.97)	0.017	0.76(0.27,2.11)	0.594
TT *vs* CC+CT	0.78(0.68,0.89)	0.000	0.65(0.49,0.86)	0.003
TT+CT *vs* CC	0.99(0.91,1.07)	0.759	1.21(0.75,1.95)	0.443
A1298C				
C *vs* A	0.92(0.85,1.00)	0.056	1.52(1.26,1.83)	0.000
CC *vs* AA	0.83(0.70,0.99)	0.042	2.43(1.38,4,28)	0.002
CC *vs* AA+AC	0.85(0.72,1.00)	0.057	1.69(0.98,2.91)	0.059
CC+AC *vs* AA	0.97(0.87,1.08)	0.557	1.93(1.47,2.49)	0.000

*HB hospital-based study; PB population-based study

95%CI=1.26-1.83, *p*=0.000; CC+AC *vs* AA: OR=1.93, 95%CI=1.47-2.49, *p*=0.000) but reduced risks were found in population-based studies (CC *vs* AA: OR=0.83, 95%CI=0.70-0.99, *p*=0.042) (Table 3).

Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias in this study. Begg's funnel plot and Egger's test were conducted to assess the potential publication bias of all included studies. The funnel plots were symmetrical, indicating that there were no evidences for obvious publication bias in the overall meta-analysis (Figure 4A, 4B). Further, Egger's test provided similar results that there was no statistical publication bias in overall meta-analysis.

Discussion

Recent evidence suggests that MTHFR polymorphisms play important roles in gastrointestinal cancer, such as gastric or esophageal cancer (Saberi et al., 2012; Qu et al., 2013; Tan et al., 2013). It has been reported that MTHFR C677T polymorphisms are associated with a reduced risk of gastric and esophageal cancer (Saberi et al., 2012; Qu et al., 2013), while MTHFR A1298C polymorphism may increase risk for esophageal cancer (Tan et al., 2013). In addition, novel studies have demonstrated that MTHFR C677T polymorphisms could influence the methylation of MGMT, a gene which was associated with TNM stages of gastric cancer and used as a predictive biomarkers for detecting of gastric cancer. Taken together, these results may further demonstrated the previous results and provided a new insight in prevention of gastric cancer (Chen et al., 2012; Gao et al., 2013; Xiong et al., 2013).

However, although Tthe relationship between MTHFR polymorphism and colon cancer risk has been extensively studied. However, no consensus has been reached. Meta-analysis is a power quantitative method by increasing the effective sample size to resolve the debated results. Therefore, we performed this meta-analysis to explore the precise association between MTHFR C677T and A1298C polymorphisms and their relationship to susceptibility for colon cancer. Overall, to our knowledge, this is the first meta-analysis to confirm the association between MTHFR polymorphism and colon cancer susceptibility.

In this meta-analysis, 13 studies with 16 373 participants indicated that MTHFR C677T polymorphism was significantly associated with a decreased colon cancer risk, especially in Non-Asians. When the analysis was performed by source of controls, this effect was observed in population-based population and hospital-based subgroup. Additionally, the MTHFR A1298C variant seemed to play a indifferent role in the risk of colon cancer and this conclusion remains among the Asians and Non-Asians as well. Interestingly, in the sub-analysis by source of controls, A1298C mutation reduced colon cancer risk in population-based population, while added colon cancer risk in hospital-based subgroup. The conflicting results mentioned above may be attributed to the sample sizes of the populations were relatively small, which may result in false-positive or false-negative outcomes.

MTHFR is a gene located on chromosome 1 at Ip36.3 with the complementary DNA sequence which is 2.2 kilo-bases long and consists of 11 exons (Goyette et al., 1998). MTHFR is an important enzyme in folate metabolism, irreversibly converting 5, 10-methylenetetrahydro- folate into 5-methyltetrahydrofolate, functions at a critical seam between DNA synthesis and DNA methylation. It is evident that MTHFR is pleomorphic and two most common single nucleotides variants within codon 677 in exon 4 (C to T, ala to val) and codon 1298 in exon 7 (A to C, glu to ala) have been found to be associated with the risk of colon cancer. The codon 677 variant, which lies within the NH2-terminal catalytic domain, encodes a thermolabile enzyme with reduced activity that leads to increased the availability of 5, 10-methylenetetrahydrofolate for DNA synthesis (Frosst et al., 1995; Taioli et al., 2009), which partially explains the reduced risk of colon cancer in subjects carrying the TT genotype in this meta-analysis.

It is inevitable to acknowledge several unavoidable limitations of our meta-analysis. Firstly, the number of published studies included in our analysis was relative small and our results might be based on unjust estimate. More studies which contained larger sample size are still needed. Secondly, only published studies were included in the meta-analysis, hence, publication bias may have occurred. Thirdly, this meta-analysis is based on unadjusted estimates, while a more precise analysis could be performed if individual data were available. Therefore, the power in the analyses was not sufficient to detect small increased risk. Finally, meta-analysis remains a retrospective research that is subject to the methodological deficiencies of the studies included.

In summary, this meta-analysis suggests that MTHFR C677T polymorphism might be protection for colon cancer susceptibility. However, the association between A1298C polymorphism and colon cancer remains unclear and further researches are needed. Given these results, the conclusion should be interpreted with caution. A large sample size including more ethnic groups with careful matching between cases and controls should be considered in future association studies to confirm the results of our meta-analysis.

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