Research Paper

The Polymorphisms in Methylenetetrahydrofolate Reductase, Methionine Synthase, Methionine Synthase Reductase, and the Risk of Colorectal Cancer

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Abstract

Polymorphisms in genes involved in folate metabolism may modulate the risk of colorectal cancer (CRC), but data from published studies are conflicting. The current meta-analysis was performed to address a more accurate estimation. A total of 41 (17,552 cases and 26,238 controls), 24(8,263 cases and 12,033 controls), 12(3,758 cases and 5,646 controls), and 13 (5,511 cases and 7,265 controls) studies were finally included for the association between *methylenetetrahydrofolate reductase* (*MTHFR*) C677T and A1289C, *methione synthase reductase* (*MTRR*) A66G, *methionine synthase* (*MTR*) A2756G polymorphisms and the risk of CRC, respectively. The data showed that the *MTHFR* 677T allele was significantly associated with reduced risk of CRC (OR = 0.93, 95%CI 0.90-0.96), while the *MTRR* 66G allele was significantly associated with increased risk of CRC (OR = 1.11, 95%CI 1.01-1.18). Sub-group analysis by ethnicity revealed that *MTHFR* C677T polymorphism was significantly associated with reduced risk of CRC in Asians (OR = 0.80, 95%CI 0.72-0.89) and Caucasians (OR = 0.84, 95%CI 0.76-0.93) in recessive genetic model, while the *MTRR* 66GG genotype was found to significantly increase the risk of CRC in Caucasians (GG vs. AA: OR = 1.18, 95%CI 1.03-1.36). No significant association was found between *MTHFR* A1298C and *MTR* A2756G polymorphisms and the risk of CRC. Cumulative meta-analysis showed no particular time trend existed in the summary estimate. Probability of publication bias was low across all comparisons illustrated by the funnel plots and Egger's test. Collectively, this meta-analysis suggested that *MTHFR* 677T allele might provide protection against CRC in worldwide populations, while *MTRR* 66G allele might increase the risk of CRC in Caucasians. Since potential confounders could not be ruled out completely, further studies were needed to confirm these results.

Key words: Colorectal cancer; Methylenetetrahydrofolate reductase; Methione synthase reductase; Methionine synthase; Folate.

Introduction

Folate is critical to one-carbon metabolism, acting as a coenzyme in nucleotide synthesis and the methylation of DNA, histones, and other proteins [1]. Accumulating evidence supports the important roles

of folate in the etiology of colorectal cancer (CRC). Methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), and methione synthase reductase (MTRR) are key enzymes involved in folate metabolism, and play essential roles in DNA synthesis, repair, and methylation. The abnormalities in these processes have been consistently observed in CRC patients, and are implicated in the pathogenesis of CRC [2].

MTHFR could convert 5,10-methylenetetrahydrofolate (5,10-MTHF), the major intracellular form of folate, to 5-methyltetrahydrofolate (5-MTHF), the major circulatory form of folate in the body (**Fig.1**) [3]. The 5-MTHF donates a methyl group to homocysteine in the generation of methionine, which then converts to S-adenosylmethionine (SAM), the universal methyl-group donor involved in methylation reaction including DNA methylation [3, 4]. The methylation of homocysteine to methionine is catalyzed by MTR using vitamin B12 as a cofactor, in which the MTR may become inactivated due to the oxidation of vitamin B12 cofactor. MTRR could catalyze reductive methylation of vitamin B12 by using SAM as a methyl donor, leading to the activation of MTR [5, 6].

Two common functional polymorphisms in the *MTHFR* gene, C677T (rs1801133) and A1298C (rs1801131), have been reported. The C677T polymorphism in exon 4 of *MTHFR* gene leads to the amino acid substitution of alanine by valine at codon 222 (A222V) which could cause a thermolabile enzyme with lower activity [7]. Homozygotes TT have approximately 30% of normal enzyme activity, whereas heterozygotes CT have approximately 60% of the normal enzyme activity, hence they tend to accumulate 5,10-methylene-THF displacing the reaction towards the DNA synthesis at the expense of the pool of methyl donors [8]. Another *MTHFR* polymorphism, A1298C polymorphism (rs1801131) in exon 7, could result in the substitution of glutamate by alanine [9]. This polymorphism could lead to a decrease of 40% in enzyme activity of the variant genotype [10]. *MTR* A2756G (rs1805087) gene polymorphism was initially thought to be associated with lower enzyme activity, leading to homocysteine elevation and DNA hypomethylation [11]. However, some other studies found a modest inverse association between 2576GG polymorphism and homocysteine levels, indicating an increased enzymatic activity of the variant genotype [12]. For the *MTRR*, the most common polymorphism is an isoleucine-to-methionine change at position 22 A66G (A66G; rs1801394), and it has demonstrated that 66GG genotype was inversely associated with plasma homocysteine levels [13].
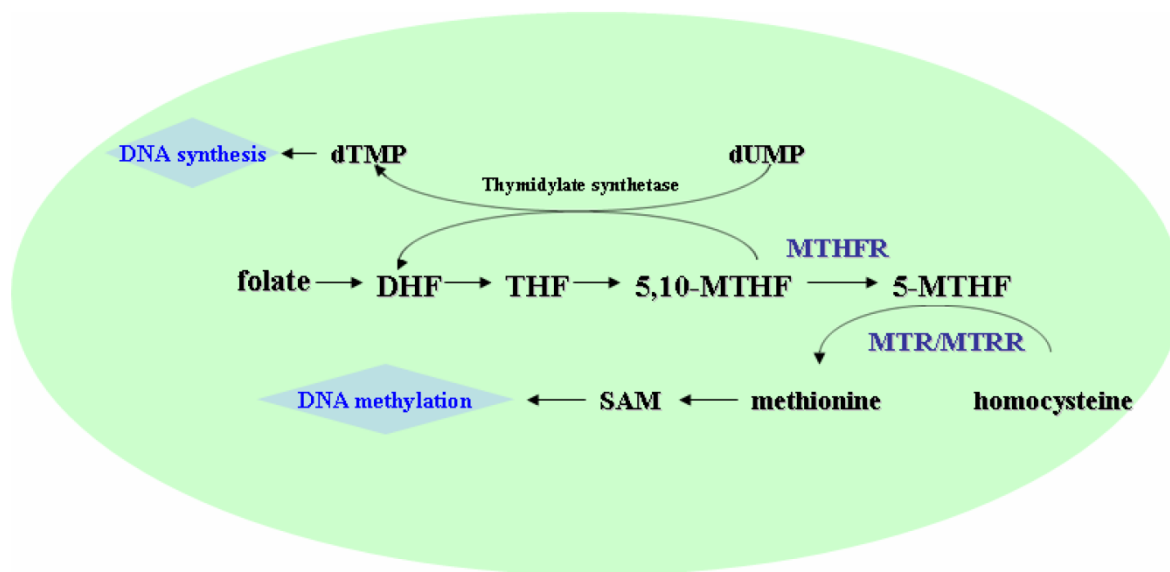

Figure 1. Schematic presentation of the folate, methionine, and homocysteine metabolism. DHF, dihydrotetrafolic acid; THF, tetrahydrofolic acid; 5,10-MTHF, 5,10-methylenetetrahydrofolate; 5-MTHF, 5-methyltetrahydrofolat; SAM, S- adenosylmethionine; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; MTRR, methione synthase reductase.

Considering the functional effects of the polymorphisms of these enzymes, it is expected that these gene polymorphisms may be associated with the CRC risks, and thus a large number of epidemiological studies have been conducted trying to clarify the above question, but obtain conflicting results. For example, some studies show a protective effect of 677TT variant against CRC [4, 14, 15], while others found no association and even deleterious effects [16-18]. Similarly, the association between the other three gene polymorphisms and the risk of CRC also remains controversial. Race, life style, and the pattern of diet may have introduced variability into the test of genetic susceptibility to disease in the different studies [19]. To clarify these issues, we performed a meta-analysis from all eligible studies, in order to provide more accurate estimate of the association of the above four gene polymorphisms and the risk of CRC.

Materials and methods

Literature and search strategy

A computerized literature search was conducted for the relevant available studies published in English from 3 databases including PubMed (1950 to 2012), ISI web of science (1975 to 2012), and Embase (1966 to 2012). The search strategy to identify all possible studies involved use of the combinations of the following key words: ("one-carbon metabolism" or "methylenetetrahydrofolate reductase" or "MTHFR" or "methionine synthase" or "MTR" or "methione synthase reductase" or "MTRR") and ("colorectal" or "colon" or "rectal") and ("cancer" or "carcinoma" or "adenocarcinoma"). The reference lists of review articles, clinical trials, and meta-analyses, were also hand-searched for the collection of other relevant studies. If more than one article were published using the same case series, only the study with largest sample size was selected. The literature search was updated on Feb 28, 2012.

Inclusion criteria

The studies included must meet the following criteria: (1) evaluating the association between *MTHFR* C677T or *MTHFR* A1289C or *MTRR* A66G or *MTR* A2756G polymorphism and the risk of CRC; (2) case-control or cohort design; (3) providing sufficient data for calculation of odds ratio (OR) with the corresponding 95% confidence interval (95%CI). When genotype frequencies and OR with 95%CI were all not available, authors were contacted to request the relevant information. All identified studies were carefully reviewed independently by two investigators to determine whether an individual study was eligible for inclusion in this meta-analysis

Data extraction

Data were extracted independently by two investigators who reached a consensus on all of the items. The following information was extracted from each study: (1) name of the first author; (2) year of publication; (3) country of origin; (4) ethnicity of the study population; (5) source of control subjects; (6) numbers of cases and controls; and (7) numbers of genotypes in cases and controls.

Statistical analysis

We use χ2 analysis with exact probability to test departure from Hardy-Weinberg equilibrium (HWE) for the genotype distribution. The association of four gene polymorphisms with CRC risk was estimated by calculating pooled ORs and 95% CI. The significance of the pooled effect size was determined by Z test. Heterogeneity among studies was assessed using Q test as well as the I^2 statistic, which was documented for the percentage of the observed between-study variability due to heterogeneity rather than chance [20]. The DerSimonian and Laird random effect model (REM) was used as the pooling method when $I^2 > 50\%$, otherwise, the Mantel-Haenszel fixed effect model (FEM) was considered to be the appropriate choice [20]. Subgroup analysis stratified by ethnicity was only considered for Asians and Caucasians as small numbers of studies were conducted in other ethnic groups. Cumulative meta-analysis was performed to assess whether the combined estimate changed in the same direction over time [21]. Influential analysis was undertaken by removing an individual study each time to check whether any of single study could bias the overall estimate [22]. An individual study was suspected of excessive influence, if the point estimate of its omitted analysis lies outside of the 95%CI of the combined analysis. Begg's funnel plots and Egger's regression test were undertaken to assess the potential publication bias [23]. Probability less than 0.05 was judged significant except for the I^2 statistic. Data analysis was performed using STATA version 11 (StataCorp LP, College Station, Texas, USA).

Results

Characteristics of studies

A total of 41 (17,552 cases and 26,238 controls), 24(8,263 cases and 12,033 controls), 12(3,758 cases and 5,646 controls), and 13 (5,511 cases and 7,265 controls) studies were finally included in the meta-analyses for the association between the *MTHFR* C677T, *MTHFR* A1298C, *MTRR* A66G, *MTR* A2756G and the risk of

CRC, respectively. Among these studies, Keku *et al.* provided data on two ethnicities [24], while Le Marchand *et al.* reported on three separate populations[25], and thus each subpopulation in these two studies were treated as a separate one. All the included studies used blood samples for the extraction of DNA, except for the study by Shannon *et al.* in which frozen tissues samples were used [26]. Genotyping was performed by using PCR-RFLP, real-time PCR, or Taqman SNP genotyping assay. These studies were performed in a wide range of geographical settings leading to a diversity of racial groups. For the *MTHFR* C677T, 20 studies recruited Caucasians [4, 14-16, 18, 24-38]; 11 studies examined individuals of Asian descent [25, 39-48]; the remaining 10 studies were on Indians, Africans, Hawaiian, or mixed population. For the *MTHFR* A1298C, *MTRR* A66G, *MTR* A2756G analyses, 12, 6, and 7 studies were conducted in Caucasians, respectively, while 5, 3, and 3 studies were in Asians. Genotype distributions in the controls of all studies were in HWE except for 8 studies for *MTHFR* C677T polymorphism [29, 31, 37, 40, 44, 48-50], 4 studies for A1298C polymorphism [14, 17, 28, 41], 1 study for *MTRR* A66G polymorphism [47], and 1 study for *MTR* A2756G polymorphism [17]. The detailed characteristics of the included studies were shown in the **supplementary Table 1**.

Quantitative data synthesis

Results of pooled analysis on the associations between *MTHFR* C677T, *MTHFR* A1298C, *MTRR* A66G, *MTR* A2756G polymorphisms and the risk of CRC were shown in **Table 1-3**, respectively. There was evidence that the T allele of *MTHFR* C677T resulted in decreased susceptibility to CRC in a worldwide population (OR = 0.93, 95%CI 0.90-0.96), as well as in Asians (OR = 0.89, 95%CI 0.84-0.94) and Caucasians (OR = 0.92, 95%CI 0.88-0.97). Mild to moderate between-study heterogeneity was found (I^2 <50%). Significant associations were also found in the genotypes contrasts in homozygous comparison (TT vs. CC: OR = 0.83, 95%CI 0.77-0.88), in the recessive model (TT vs. TC+CC: OR = 0.84, 95%CI 0.79-0.89), and in the dominant model (TT+TC vs. CC: OR = 0.95, 95%CI 0.91-0.99), except for the comparison of CT vs. CC (OR = 0.98, 95%CI 0.94-1.02) in the worldwide populations (**Table 1**). The sub-group analysis by ethnicity also revealed significant association in Asians and Caucasians in the above genotype contrasts except for the homozygous contrast in Caucasians. (TT vs. CC, OR = 0.85, 95%CI 0.72-1.01) (**Fig.2**). Sensitivity analysis was performed by excluding 8 studies deviated from HWE [29, 31, 37, 40, 44, 48-50]; however, the results were not materially altered in either genetic model (**Table 1**).

In contrast to the significant association between *MTHFR* C677T and the risk of CRC, the *MTHFR* A1298C polymorphism was found to be not significantly related with the risk of CRC. As shown in **Table 2**, a marginal association was found in the allele comparison (G vs. A: OR = 0.96, 95%CI =0.91-1.00, *P* = 0.50), and in the homozygous genotypes comparison (GG vs. AA: OR = 0.86, 95%CI = 0.78-0.96), and in the recessive model (GG vs. GA+AA: OR = 0.86, 95%CI = 0.78-0.95). The pooled results were not significantly changed after exclusion of studies deviated from HWE in every contrast (**Table 2**). However, when we further excluded the studies by Wang *et al.* and Keku *et al.*, in which the 95%CI did not overlap the lines of the pooling results, the significant associations were all disappeared (G vs. A: OR = 0.97, 95%CI =0.93-1.02; GG vs. AA: OR = 0.92, 95%CI = 0.82-1.03; GG vs. GA+AA: OR = 0.91, 95%CI = 0.81-1.01).

This meta-analysis also provided supporting evidence for the association of *MTRR* A66G polymorphism and CRC risk. As shown in **Table 3**, G allele of *MTRR* A66G resulted in increased susceptibility to CRC in a worldwide population (OR = 1.11, 95%CI 1.01-1.18), as well as in Caucasians (OR = 1.09, 95%CI 1.01-1.16), but not in Asians (OR = 1.12, 95%CI 0.95-1.32). No between-study heterogeneity was found in these comparison (I^2 =0.0%). Regarding the genotypes, there was also significant association in homozygous contrast (GG vs. AA) in the worldwide populations (OR = 1.24, 95%CI 1.09-1.40) as well as in the Caucasians (OR = 1.18, 95%CI 1.03-1.36), but not in Asians (OR = 1.40, 95%CI 0.71-2.77) (**Fig.3**). However, no significant association was found in Asians or Caucasians in other genetic comparisons (**Table 3**). Furthermore, the pooled results were not significantly altered after excluding one study by Mastuo *et al.* [47], which was deviated form HWE (data was not shown).

No significant association was found for the *MTR* A2756G polymorphism and the risk of the CRC (**Table 3**). The polled results were also not significantly altered after exclusion of the study deviated from HWE (data was not shown).

Influence analysis and cumulative analysis

After excluding studies that deviated from HWE in controls, and those in which 95%CI did not overlap the lines of the pooling results, no other studies were found to significantly influence the pooled effects in each genetic model. In the cumulative meta-analysis, no particular time trend was found in the summary estimate (data was not shown).

Publication bias

Funnel plots were generated to assess publication bias. The Egger's test was performed to statistically evaluate funnel plot symmetry. The results suggested no publication bias for the association of the *MTHFR* C677T, *MTHFR* A1298C, *MTRR* A66G, *MTR* A2756G polymorphisms and the risk of CRC ($P_{\text{Egger test}}$ = 0.474, 0.922, 0.118, and 0.201, respectively). (**Fig.4**).

Table 1 Ethnicity-stratified pooled measures on the association between *MTHFRC677T* polymorphism and colorectal cancer.

Contrast	Ethnicity	All relevant articles were included (n = 41)					Articles deviated for HWE were excluded (n=33)[a]				
		OR	95%CI	Statistical model	I^2(%)	p value [b]	OR	95%CI	Statistical model	I^2(%)	p value [b]
T *vs.* C	Asian	0.89	(0.84-0.94)**	FEM	47.6%	0.039	0.91	(0.85-0.97)**	FEM	25.2%	0.228
	Caucasian	0.92	(0.88-0.97)**	FEM	46.4%	0.012	0.93	(0.88-0.97)**	FEM	49.1%	0.012
	others	0.98	(0.93-1.04)	FEM	5.3%	0.391	0.98	(0.92-1.04)	FEM	17.1%	0.295
	All	0.93	(0.90-0.96)**	FEM	43.3%	0.002	0.94	(0.91-0.97)**	FEM	38.3%	0.013
TT *vs.* CC	Asian	0.76	(0.68-0.86)**	FEM	34.1%	0.126	0.80	(0.70-0.92)**	FEM	29.3%	0.194
	Caucasian	0.85	(0.72-1.01)	REM	50.8%	0.005	0.86	(0.72-1.03)**	REM	53.1%	0.005
	Others	0.90	(0.80-1.02)	FEM	25.9%	0.214	0.90	(0.78-1.03)	FEM	35.0%	0.149
	All	0.83	(0.77-0.88)**	FEM	42.3%	0.003	0.84	(0.78-0.90)**	FEM	42.9%	0.005
CT *vs.* CC	Asian	0.94	(0.86-1.02)	FEM	0.0%	0.711	0.95	(0.86-1.04)	FEM	0.0%	0.715
	Caucasian	0.95	(0.90-1.02)	FEM	39.9%	0.034	0.95	(0.89-1.01)	FEM	46.6%	0.018
	others	1.04	(0.97-1.13)	FEM	0.0%	0.984	1.03	(0.95-1.12)	FEM	0.0%	0.977
	All	0.98	(0.94-1.02)	FEM	13.0%	0.241	0.97	(0.93-1.02)	FEM	17.8%	0.186
TT *vs.* TC+CC	Asian	0.80	(0.72-0.89)**	FEM	19.5%	0.258	0.83	(0.74-0.94)**	FEM	13.5%	0.325
	Caucasian	0.84	(0.76-0.93)**	REM	53.7%	0.002	0.89	(0.74-1.06)	REM	56.2%	0.002
	others	0.88	(0.78-0.99)*	FEM	20.9%	0.257	0.88	(0.78-1.01)	FEM	30.1%	0.188
	All	0.84	(0.79-0.89)**	FEM	39.8%	0.006	0.85	(0.80-0.91)**	FEM	41.8%	0.007
TT+CT *vs.* CC	Asian	0.89	(0.82-0.97)**	FEM	21.2%	0.241	0.91	(0.83-1.00)*	FEM	0.0%	0.446
	Caucasian	0.93	(0.88-0.99)*	FEM	39.1%	0.039	0.93	(0.87-0.99)*	FEM	44.7%	0.024
	others	1.02	(0.95-1.09)	FEM	0.0%	0.839	1.01	(0.93-1.09)	FEM	0.0%	0.767
	All	0.95	(0.91-0.99)**	FEM	27.7%	0.056	0.95	(0.91-0.99)*	FEM	26.3%	0.086

[a] 8 studies by Sameer, 2011; by Mohebbi,2008; Zeybek, 2007; Koushik, 2006; Le Marchand, 2005; Jiang, 2005; Park, 1999, and Ma, 1997, were excluded as they were deviated from Hardy-Weinberg equilibrium; [b] p value for heterogeneity based on Q test; FEM, fixed effect model; REM, random effect model; *p<0.05; **p<0.01.

Table 2 Ethnicity-stratified pooled measures on the association between *MTHFR A1298C* polymorphism and colorectal cancer.

Contrast	Ethnicity	All relevant articles were included (n=24)					Articles deviated for HWE were excluded (n=20)[a]				
		OR	95%CI	Statistical model	I^2(%)	p value [b]	OR	95%CI	Statistical model	I^2(%)	p value [b]
C *vs.* A	Asian	0.92	(0.80-1.06)	FEM	0.0%	0.680	0.90	(0.77-1.05)	FEM	0.0%	0.678
	Caucasian	0.99	(0.93-1.05)	FEM	9.6%	0.351	0.99	(0.93-1.05)	FEM	25.9%	0.206
	others	0.93	(0.86-0.99)*	FEM	48.8%	0.068	0.91	(0.85-0.98)*	FEM	43.1%	0.118
	All	0.96	(0.91-1.00)*	FEM	18.6%	0.207	0.95	(0.91-0.99)*	FEM	25.4%	0.146
CC *vs.* AA	Asian	0.76	(0.49-1.19)	FEM	0.0%	0.488	0.75	(0.44-1.26)	FEM	0.0%	0.871
	Caucasian	0.93	(0.81-1.07)	FEM	11.3%	0.334	0.93	(0.81-1.08)	FEM	19.9%	0.260
	others	0.79	(0.67-0.93)**	FEM	12.1%	0.337	0.78	(0.66-0.92)**	FEM	14.4%	0.322
	All	0.86	(0.78-0.96)**	FEM	0.0%	0.488	0.86	(0.77-0.96)**	FEM	8.6%	0.349
CA *vs.* AA	Asian	0.95	(0.80-1.13)	FEM	0.0%	0.470	0.91	(0.75-1.09)	FEM	0.0%	0.618

Contrast	Ethnicity	All relevant articles were included (n=24)					Articles deviated for HWE were excluded (n=20)[a]				
		OR	95%CI	Statistical model	I^2(%)	p value [b]	OR	95%CI	Statistical model	I^2(%)	p value [b]
	Caucasian	1.03	(0.95-1.12)	FEM	0.0%	0.610	1.03	(0.94-1.12)	FEM	0.0%	0.567
	others	0.98	(0.89-1.08)	FEM	23.4%	0.251	0.97	(0.88-1.07)	FEM	7.6%	0.368
	All	1.00	(0.94-1.06)	FEM	0.0%	0.555	0.99	(0.93-1.06)	FEM	0.0%	0.617
CC *vs.* CA+AA	Asian	0.77	(0.49-1.19)	FEM	0.0%	0.953	0.77	(0.46-1.29)	FEM	0.0%	0.878
	Caucasian	0.91	(0.80-1.04)	FEM	0.0%	0.444	0.92	(0.80-1.06)	FEM	1.2%	0.427
	others	0.80	(0.68-0.94)**	FEM	0.0%	0.670	0.79	(0.67-0.93)**	FEM	0.0%	0.617
	All	0.86	(0.78-0.95)**	FEM	0.0%	0.781	0.86	(0.78-0.95)**	FEM	0.0%	0.689
CC+CA *vs.* AA	Asian	0.93	(0.79-1.10)	FEM	0.0%	0.533	0.89	(0.74-1.07)	FEM	0.0%	0.622
	Caucasian	1.01	(0.93-1.09)	FEM	0.0%	0.472	1.01	(0.93-1.10)	FEM	11.2%	0.340
	others	0.95	(0.86-1.04)	FEM	43.4%	0.101	0.93	(0.85-1.03)	FEM	35.2%	0.173
	All	0.98	(0.92-1.03)	FEM	10.8%	0.311	0.96	(0.91-1.02)	FEM	13.2%	0.290

[a] 4 studies by Guimaraes, 2011, Fernandez-Peralta, 2010, Sharp, 2008, and Chang, 2007, were excluded as they were deviated from Hardy-Weinberg equilibrium;

[b] p value for heterogeneity based on Q test; FEM, fixed effect model; *p<0.05; **p<0.01.

Table 3 Ethnicity-stratified pooled measures on the association between *MTRR A66G /MTR A2756G* polymorphism and colorectal cancer.

Contrast	Ethnicity	*MTRR A66G* polymorphism and CRC (n=12)					*MTR A2756G* polymorphism and CRC (n=13)				
		OR	95%CI	Statistical model	I^2(%)	p value [a]	OR	95%CI	Statistical model	I^2(%)	p value [a]
G *vs.* A	Asian	1.12	(0.95-1.32)	FEM	0.0%	0.552	1.03	(0.87-1.21)	FEM	0.0%	0.713
	Caucasian	1.09	(1.01-1.16)*	FEM	0.0%	0.434	0.96	(0.85-1.10)	REM	55.2%	0.037
	others	1.25	(1.03-1.50)*	FEM	0.0%	0.697	1.19	(0.95-1.48)	FEM	0.0%	0.670
	All	1.11	(1.04-1.18)**	FEM	0.0%	0.661	0.98	(0.92-1.04)	FEM	36.3%	0.092
GG *vs.* AA	Asian	1.40	(0.71-2.77)	REM	58.3%	0.091	1.30	(0.81-2.08)	FEM	0.0%	0.495
	Caucasian	1.18	(1.03-1.36)*	FEM	23.7%	0.256	0.83	(0.67-1.03)	FEM	48.1%	0.073
	Others	1.63	(1.08-2.45)*	FEM	0.0%	0.693	1.49	(0.81-2.72)	FEM	0.0%	0.876
	All	1.24	(1.09-1.40)**	FEM	25.1%	0.197	0.94	(0.78-1.14)	FEM	34.4%	0.107
GA *vs.* AA	Asian	1.03	(0.82-1.29)	FEM	0.0%	0.559	0.96	(0.79-1.18)	FEM	0.0%	0.939
	Caucasian	1.15	(0.93-1.42)	REM	57.6%	0.038	0.97	(0.89-1.06)	FEM	0.0%	0.426
	Others	1.14	(0.86-1.49)	FEM	0.0%	0.983	1.15	(0.87-1.52)	FEM	0.0%	0.679
	All	1.09	(0.99-1.21)	FEM	17.5%	0.272	0.98	(0.91-1.06)	FEM	0.0%	0.768
GG *vs.* GA+AA	Asian	1.39	(0.64-3.01)	REM	69.7%	0.037	1.32	(0.83-2.10)	FEM	0.0%	0.498
	Caucasian	1.10	(0.99-1.22)	FEM	0.0%	0.980	0.84	(0.67-1.04)	FEM	39.7%	0.127
	others	1.56	(1.08-2.25)*	FEM	0.0%	0.651	1.44	(0.79-2.60)	FEM	0.0%	0.883
	All	1.15	(1.04-1.26)*	FEM	13.0%	0.317	0.95	(0.79-1.14)	FEM	27.3%	0.169
GG+GA *vs.* AA	Asian	1.09	(0.88-1.34)	FEM	0.0%	0.981	0.99	(0.82-1.21)	FEM	0.0%	0.868
	Caucasian	1.17	(0.96-1.42)	REM	55.2%	0.048	0.96	(0.88-1.04)	FEM	37.4%	0.143
	others	1.23	(0.95-1.59)	FEM	0.0%	0.917	1.19	(0.91-1.56)	FEM	0.0%	0.680
	All	1.14	(1.03-1.25)*	FEM	7.4%	0.373	0.98	(0.91-1.05)	FEM	8.1%	0.365

[a] p value for heterogeneity based on Q test; FEM, fixed effect model. REM, random effect model; *p<0.05; **p<0.01.
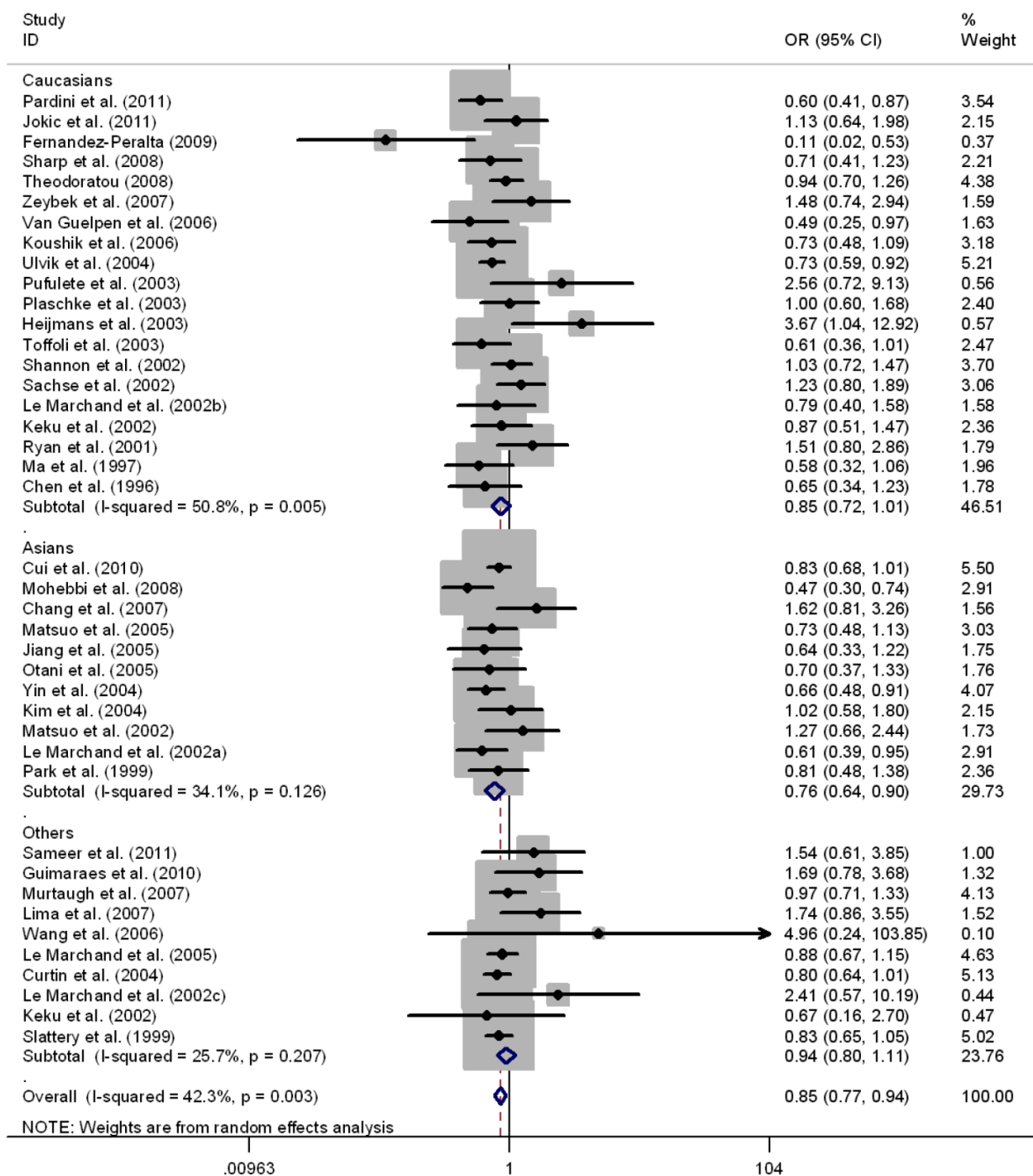
Figure 2. Meta-analysis for *MTHFR* C677T polymorphism and CRC in Caucasian, Asian, and the worldwide population (TT vs. CC). Each study was shown by a point estimate of the effect size (OR) (size inversely proportional to its variance) and its 95% confidence interval (95%CI) (horizontal lines). The whit diamond denotes the pooled OR.
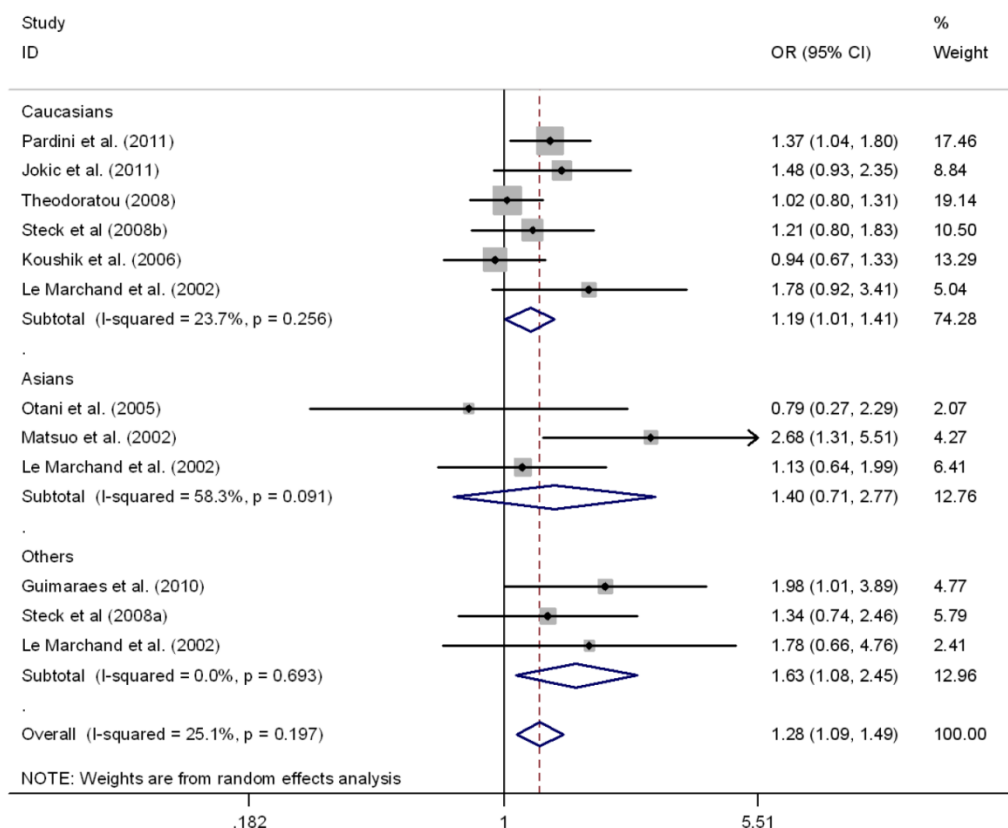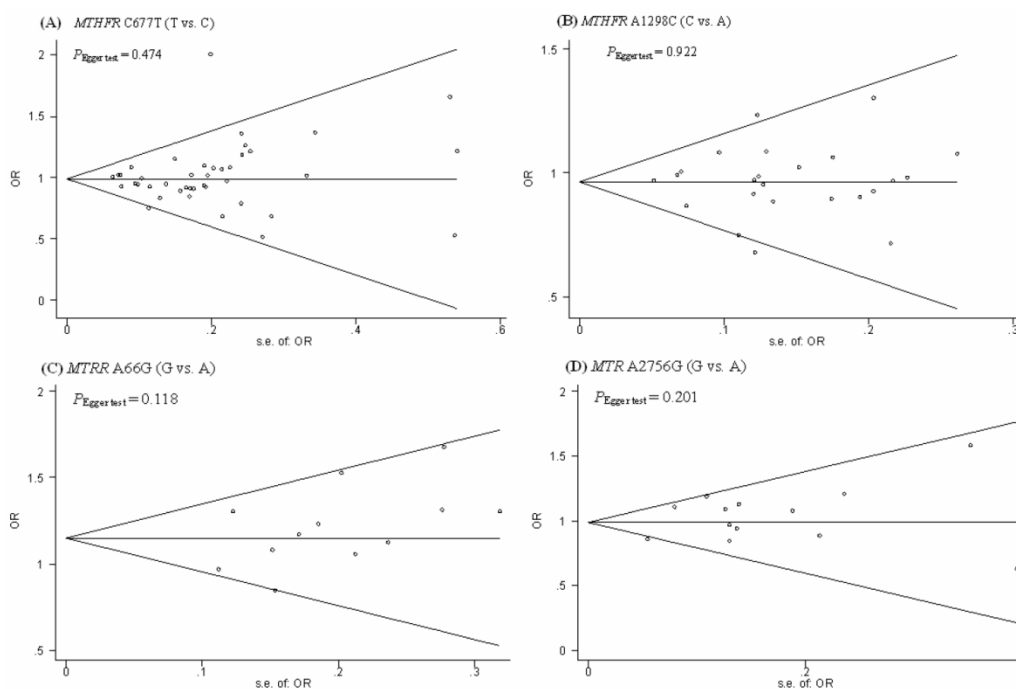
Figure 3. Meta-analysis for *MTRR* A66G polymorphism and CRC in Caucasian, Asian, and the worldwide population (GG vs. AA). Each study was shown by a point estimate of the effect size (OR) (size inversely proportional to its variance) and its 95% confidence interval (95%CI) (horizontal lines). The whit diamond denotes the pooled OR.

Figure 4. Begg's funnel plot with the Egger's test for publication bias of *MTHFR* C677T and A1289C, *MTRR* A66G, *MTR* A2756G polymorphism and the risk of CRC. The horizontal line in the funnel plot indicates the fixed-effects summary estimate, whereas the diagonal lines pseudo-95% CI limits about the effect estimate. In the absence of publication bias, studies will be distributed symmetrically above and below the horizontal line.

Discussion

CRC is the third most common cause of cancer-related mortality in the western world, and it represents the second most common cause of cancer death in the United States [51]. The incidence of CRC varies substantially worldwide, with high rates in western countries and low rates in African and Asian countries in general [52]. Over the past decades, the roles of folate and genetic polymorphisms of enzymes involved in folate metabolism have attracted considerable interest in epidemiological research on this cancer type. Among these enzymes, MTHFR, MTR, and MTRR, are the three mostly investigated ones in the literature. Unfortunately, conflicting results were obtained ranging from strong links to no association. The divergent results regarding the effects of these genetic polymorphisms upon CRC risk may be attributed to the differences in racial origin of the population, the lifestyle, and the pattern of diet in distinct countries [17]. Because of the above-mentioned conflicting results from relatively small studies underpowered to detect the effects, a meta-analysis should be an appropriate approach to obtain a more definitive conclusion.

In the current study, we examined the association of *MTHFR* C677T and A1298C, *MTR*R A66G, and *MTR* A2756G polymorphisms with the susceptibility to CRC, with a total of 41, 24, 12, and 13 studies included, respectively. The data clearly showed that the T allele of C677T polymorphism in *MTHFR* gene was significantly associated with reduced risk of CRC, while the G allele of A66G polymorphism in *MTRR* gene might be significantly associated with increased risk of CRC (**Table 1 and 3**). The sub-group analysis by ethnicity revealed that homozygosity for the T allele (*MTHFR* 677TT genotype) is associated with significantly reduced risk of CRC in Asians and Caucasians, while the *MTR* 66GG genotype was found to be significantly increased the risk of CRC in Caucasians but not in Asians. However, no significant association was found between the *MTHFR* A1298C and *MTR* A2756G polymorphisms and the risk of CRC (**Table 2 and 3**). Although a marginal association was found in the allele and genotypes contrasts of *MTHFR* A1298C (C vs. A, CC vs. AA, and CC vs. CA+AA), the signification was all disappeared after exclusion of one study, in which the 95%CI did not overlap the lines of the pooled results. After excluding studies that deviated from HWE in controls, and those in which 95%CI did not overlap the lines of the pooling results, no other studies were found to significantly influence the pooled effects in each genetic model. Cumulative meta-analysis showed no particular time trend existed in the summary estimate. Furthermore, no potential publication bias was detected by funnel plots and Egger's regression test. These data indicated the robustness of the summary estimate deserved from this study.

To the best of our knowledge, this is the first meta-analysis addressing the associations between the four common polymorphisms of three critical enzymes involved in folate metabolism and the risk of CRC in one study. From 2005 to now, a total of 5 meta-analyses about the association between *MTHFR* C677T and the risk of CRC have been conducted [52-56]. The most recently one conducted by Taioli *et al*. included 29 studies; 28 of those were included in our meta-analysis, which also included an additional 11 studies primarily published between 2008 and 2012. The pooled results of the current meta-analysis were similar to those by Taioli *et al*. The pooled OR for genotype TT (vs. CC) was 0.83 (95%CI: 0.77, 0.90), while those were 0.83 (95%CI: 0.74, 0.94) and 0.80 (95%CI: 0.67, 0.96) in Caucasians and Asians, respectively [53]. Similarly, the pooled ORs in our study were 0.83(95%CI: 0.77, 0.88), 0.85 (95%CI: 0.72, 1.01), and 0.76 (95%CI: 0.68, 0.86), respectively. The meta-analysis conducted by Huang *et al*. included 14 studies for the analysis of the association of *MTHFR* A1298C and the risk of CRC [55]. They found that a significantly decreased risk of CRC for 1298C polymorphism in a recessive genetic model in worldwide populations (OR = 0.81, 95%CI 0.70-0.94) and Caucasians (OR = 0.75, 95%CI 0.57-0.99). However, although a marginal association was found in the allele and genotypes contrast of *MTHFR* A1298C (C vs. A, CC vs. AA, and CC vs. CA+AA), the signification all disappeared after exclusion of the study by Wang *et al*. and Keku *et al*. [24, 57], in which the 95%CI did not overlap the lines of the pooled results (G vs. A: OR = 0.97, 95%CI =0.93-1.02; GG vs. AA: OR = 0.92, 95%CI = 0.82-1.03; GG vs. GA+AA: OR = 0.91, 95%CI = 0.81-1.01). The discrepancy between our study and the study by Huang *et al*. might be explained by the addition of 12 studies and the different methodology of the two studies by Wang *et al*. and Keku *et al*. [24, 57]. This meta-analysis also provided supporting evidence for the association of *MTRR* A66G polymorphism and the risk of CRC. G allele of *MTRR* A66G resulted in increased susceptibility to CRC in a worldwide population (OR = 1.11, 95%CI 1.01-1.18), as well as in Caucasians (OR = 1.09, 95%CI 1.01-1.16), but not in Asians (OR = 1.12, 95%CI 0.95-1.32). In contrast, no significant association was found for the *MTR* A2756G polymorphism and the risk of CRC.

Reasons for the conflicting results obtained from different studies about the association between the

polymorphisms of the three investigated genes involved in folate metabolism and the risk of CRC may be attributed to the genetic heterogeneity in different populations and the clinical heterogeneity in different studies. CRC is well-known as a multifaceted disorder influenced by both genetic and environmental factors. The differences in age, gender, and lifestyle may lead to different results. In the current meta-analysis, most of the studies enrolled subjects of both gender, while some others investigated the association in male patients [37]. In addition, several studies have demonstrated the associations between genotypes *MTHFR* 677CT+TT and *MTR* 2756AG+GG genotypes with an early disease onset (<50 years of age) [17, 19]. Furthermore, folate deficiency which was seen in many studies, might also affect the activity of enzymes involved in DNA synthesis, methylation, and repair pathways, leading to the hypomethylation of DNA and the activation of proto-oncogene, as well as the inducing uracil misincorporation during DNA synthesis, which may also contribute to the discrepancy of different studies [58]. In fact, the study by Chen *et al.* have suggested that dietary methyl supply was particularly critical among patients with *MTHFR* CC genotypes, which may be at a reduced risk of CRC probably as higher levels of 5,10-MTHF may prevent imbalances of nucleotide pools during DNA synthesis [38]. Moreover, the association of alcohol consumption with CRC risk has been related to its anti-folate effects and subsequent effects on DNA methylation [59]. Thus, age, gender, and diet particularly folate and alcohol intake, may modify the effects of these gene polymorphisms and the risk of CRC.

Despite the clear strengths of our study such as the larger sample size comparing with the previous individual ones, however, it does have some limitations. First, the present meta-analysis was based primarily on unadjusted effect estimates and CIs (since most studies did not provide the adjusted OR and 95%CI controlling for potential confounding factors), so the effect estimates were relatively imprecise. If individual data were available, adjusted Ors could be obtained to give a more precise analysis. Second, the effects of gene-gene and gene-environment interactions were not addressed in this meta-analysis, and thus the potential roles of the above gene polymorphism may be masked or magnified by other gene-gene/gene-environment interactions. Thirdly, we did not perform stratification analysis by age, gender, drinking status, serum folate levels, etc, which might induce confounding bias. Finally, although the funnel plot and Egger's test showed no publication bias, selection bias may also exist because only published studies were retrieved.

In summary, the current meta-analysis systematically analyzed the association between the risk of CRC and four common polymorphisms of three important genes involved in folate metabolism. The data showed that *MTHFR* 677T allele might provide protection against CRC in worldwide populations, while *MTRR* 66G allele might increase the risk of CRC in Caucasians but not in Asians. In contrast, *MTHFR* A1298C and *MTR* A2756G polymorphism were unlikely to be related with the risk of CRC. Since potential confounders could not be ruled out completely in this meta-analysis, further studies were needed to confirm these results.

Supplementary Material

Supplementary Table 1. Characteristics of studies included in the meta-analysis.
http://www.biolsci.org/v08p0819s1.pdf

Competing Interests

The authors have declared that no competing interest exists.

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