

RESEARCH COMMUNICATION

MTHFR C677T Polymorphism and Colorectal Cancer Risk in Asians, a Meta-analysis of 21 Studies

Zhen Yang*, Xie-Fu Zhang, Hong-Xiang Liu, Yong-Shun, Hao, Chun-Lin Zhao

Abstract

Background: Previous studies concerning the association between methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and colorectal cancer risk in Asian populations generated conflicting results. A meta-analysis was therefore performed to allow a more reliable estimate of any link. **Methods:** Relevant studies concerning the association between the MTHFR C677T polymorphism and risk of colorectal cancer were included into this meta-analysis. The quality of the studies was assessed according to a predefined scale. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined for this gene-disease association using fixed or random effect models according to the heterogeneity between included studies. **Results:** Finally, 21 studies with a total of 6692 cases and 8266 controls were included. Meta-analyses showed that there was an obvious association of the MTHFR 677T allele with decreased risk of colorectal cancer (OR = 0.91, 95% CI=0.85-0.98, P=0.011). Subgroup analyses by country further identified this association, with dietary folate as the main source of heterogeneity. **Conclusion:** The MTHFR 677T allele is associated with a lower risk of colorectal cancer in Asian populations, and there is effect modification by population plasma folate.

Keywords: MTHFR C677T - colorectal cancer - polymorphism - meta-analysis

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females with over 1.2 million new cancer cases and 608,700 deaths estimated to have occurred in the world (Jemal et al., 2010; Jemal et al., 2011). Thus, CRC still is a serious fatal disease worldwide and has caused serious damage to human health. As a complex and multi-factorial process, the colorectal carcinogenesis is still not fully understood. Epidemiological studies have revealed that smoking, diets and other environmental risk factors play important roles in the development of CRC. However, only a small proportion of individuals exposed to the known risk factors develop CRC, while many cases develop CRC among individuals without those risk factors, which suggest genetic factors also play an important role in the colorectal carcinogenesis (Markowitz et al., 2009).

Methylenetetrahydrofolate reductase (MTHFR) C677T in the gene encoding the MTHFR enzyme, which converts dietary folate to its active cofactor in Hcy catabolism, has been studied as a candidate genetic risk factor for CRC risk (Goyette et al., 1994). As T allele dose increases, this functional polymorphism causes a graded elevation in tHcy in the mild-moderate range, most pronounced in individuals with low dietary folate consumption (Frosst et al., 1995). Thus, considering the potential influence on

folate metabolism, many epidemiological studies have explored the association between the MTHFR C677T polymorphism and CRC risk, but the results are conflicting (Park et al., 1999; Matsuo et al., 2002; 2005; Kim et al., 2004; Yin et al., 2004; Otani et al., 2005; Chang et al., 2007; Cao et al., 2008; Cui et al., 2010; Promthet et al., 2010; Kim et al., 2011; Zhu et al., 2011). Small genetic association studies have various designs, different methodology and insufficient power, and could inevitably increase the risk that chance could be responsible for their conclusions, while combining data from all eligible studies by meta-analysis has the advantage of reducing random error and obtaining precise estimates for some potential genetic associations (Egger et al., 1997). Therefore, to address this controversial issue, we performed a meta-analysis of all available studies to clarify the effects of MTHFR C677T polymorphism on CRC risk.

Materials and Methods

Identification and eligibility of relevant studies

We searched PubMed, Embase and CBM database using the following search strategy ('colorectal carcinoma' or 'colorectal cancer' or 'colon cancer' or 'rectal cancer') and ('Methylenetetrahydrofolate reductase' or 'MTHFR' or 'C677T') and ('polymorphism' or 'polymorphisms' or 'mutation' or 'mutations') for published papers. The

Department of Colorectal Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China *For correspondence: yangzhen@yahoo.cn

language of the papers was not restricted. All references cited in these studies and previously published review articles were retrieved for additional eligible studies. The following criteria were used to select the eligible studies, (1) a case-control study on the association between the MTHFR C677T polymorphism and CRC risk; (2) identification of CRC was confirmed histologically or pathologically; (3) an available genotype or allele frequency for estimating an odds ratio (OR) with a 95% confidence interval (CI). When the same authors reported two or more publications on possibly the same patient populations, only the most recent or complete study was included into this meta-analysis. The major reasons for exclusion of studies were, (1) family studies; (2) case only studies; (3) review papers; (4) containing overlapping data.

Data extraction

Two reviewers independently evaluated the final articles included into this meta-analysis, and disagreements were resolved by reaching a consensus among all authors. Data retrieved from the articles included the following, first author's name, publication year, country of origin, source of controls, eligible and genotyped cases and controls, the number for each MTHFR C677T genotype, and the allele frequency of MTHFR C677T.

Quality score assessment

The quality of the studies was also independently assessed by the same two reviewers according to the predefined scale for quality assessment (Table 1) (Jiang et al., 2010; Jiang et al., 2011). These scores were based on both traditional epidemiological considerations and cancer genetic issues. Any disagreement was resolved by discussion between the two reviewers. Total scores ranged from 0 (worst) to 15 (best). Reports scoring < 10 were classified as "low quality", and those ≥ 10 as "high quality".

Statistical methods

For the control group of each study, the distributions of genotypes were tested for Hardy-Weinberg equilibrium (HWE) using the Chi-square test. If controls of studies were found not to be in HWE, sensitivity analyses were performed with and without these studies to test the robustness of the findings. The strength of association between MTHFR C677T polymorphism and CRC risk was estimated by Odds ratios (ORs) with 95% confidence intervals (CIs). Four different comparison models of ORs were calculated, the allele model (T vs. C), the Homozygote comparison model (TT versus CC), the Recessive genetic comparison model (TT versus TC+CC), and the Dominant genetic comparison model (TT + TC versus CC). The I^2 index expressing the percentage of the total variation across studies due to heterogeneity was calculated to assess the between-study heterogeneity. I^2 values of 25%, 50%, and 75% were used as evidence of low, moderate, and high heterogeneity, respectively (Higgins et al., 2003). If moderate or high heterogeneity existed, the random effects model (the DerSimonian and Laird method), which yields wider confidence intervals, was adopted to calculate the overall OR value (Der

Simonian et al., 1986). Otherwise, the fixed effects model (the Mantel-Haenszel method) was used (Mantel et al., 1959). To study the source of between-study heterogeneity, meta-regression was also performed (Thompson et al., 2002). One-way sensitivity analyses were performed to assess the stability of the meta-analysis results (A et al., 1999). Funnel plots and Egger's linear regression test were used to assess evidence for potential publication bias (Egger et al., 1997; Stuck et al., 1998). The analysis was conducted using STATA (Biostat, NJ, USA). All P values were two-sided and a P value of less than 0.05 was deemed statistically significant.

Results

Characteristics of studies

298 records were initially identified by the search. After discarding overlapping references and those which clearly did not meet the criteria, 22 studies were further assessed for eligibility (Park et al., 1999; Matsuo et al., 2002; Huang et al., 2003; Jiang et al., 2004; Kim et al., 2004; Yin et al., 2004; Jiang et al., 2005; Matsuo et al., 2005; Miao et al., 2005; Otani et al., 2005; Chang et al., 2007; Jin et al., 2007; Cao et al., 2008; Zhang et al., 2008; Cui et al., 2010; Promthet et al., 2010; Yang et al., 2010; Zhu et al., 2010; Kang et al., 2011; Kim et al., 2011; Zhu et al., 2011; Kim et al., 2012). After reviewing each original paper and extracting data, one study was excluded for overlapping data (Jiang et al., 2004). Finally, 21 case-control studies with a total of 6,692 cases and 8266 controls were included into this meta-analysis (Park et al., 1999; Matsuo et al., 2002; Huang et al., 2003; Jiang et al., 2004; Kim et al., 2004; Yin et al., 2004; Jiang et al., 2005; Matsuo et al., 2005; Miao et al., 2005; Otani et al., 2005; Chang et al., 2007; Jin et al., 2007; Cao et al., 2008; Zhang et al., 2008; Cui et al., 2010; Promthet et al., 2010; Yang et al., 2010; Zhu et al., 2010; Kang et al., 2011;

Table 1. Scale for Quality Assessment

Criterion	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
Hospital-based (cancer-free patients)	1
Not described	0
Specimens used for 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	
>1,000	3
>500 and <1,000	2
>200 and <500	1
<200	0

Table 2. Characteristics of 21 Case-control Studies Included in this Meta-analysis

Study [Ref.]	Ethnicity	Country	Case group			Control group			P _{HWE}	Quality score
			TT	TC	CC	TT	TC	CC		
Park KS 1999 (Park 1999)	East Asian	Korea	28	107	65	74	246	140	<0.05	8
Matsuo K 2002 (Matsuo 2002)	East Asian	Japan	22	81	39	36	124	81	0.3	12
Yin G 2004 (Yin 2004)	East Asian	Japan	85	330	270	133	367	278	0.53	13
Kim DH 2004 (Kim 2004)	East Asian	Korea	35	122	86	33	109	83	0.77	14
Matsuo K 2005 (Matsuo 2005)	East Asian	Japan	36	114	106	134	348	289	0.1	13
Otani T 2005 (Otani 2005)	East Asian	Japan	25	49	32	57	114	51	0.68	13
Chang SC 2007 (Chang 2007)	East Asian	China	24	86	85	16	87	92	0.47	13
Cao HX 2008 (Cao 2008)	East Asian	China	52	154	109	66	183	121	0.82	13
Promthet SS 2010 (Promthet 2010)	East Asian	Thailand	0	26	104	5	31	94	0.24	10
Cui LH 2010 (Cui 2010)	East Asian	Korea	284	923	622	297	863	540	0.13	13
Zhu Q 2011 (Zhu 2011)	East Asian	China	15	42	29	10	41	49	0.74	13
Kim JW 2011 (Kim 2011)	East Asian	Korea	7	30	30	17	21	15	0.13	13
Kang BS 2011 (Kang 2011)	East Asian	Korea	34	134	87	65	238	145	<0.05	7
Kim J 2012 (Kim 2012)	East Asian	Korea	129	393	265	162	289	205	<0.05	8
Yang XX 2010 (Yang 2010)	East Asian	China	22	61	58	28	75	62	0.53	11
Zhu F 2010 (Zhu 2010)	East Asian	China	26	102	88	8	53	50	0.23	11
Zhang YL 2008 (Zhang 2008)	East Asian	China	67	136	97	69	139	91	0.26	10
Jin XX 2007 (Jin 2007)	East Asian	China	37	154	143	102	236	162	0.35	11
Miao XP 2005 (Miao 2005)	East Asian	China	58	87	53	86	201	133	0.53	12
Jiang QT 2004 (Jiang 2005)	East Asian	China	15	59	51	63	144	133	<0.05	8
Huang P 2003 (Huang 2003)	East Asian	China	6	40	36	9	33	40	0.58	11

Table 3. Summary of Pooled Odds Ratios (OR) with Confidence Interval (CI) in the Meta-analysis

Comparison Model	Studies (cases / controls)	Odds Ratio		M*	I ²
		OR[95%CI]	P _{OR}		
Analysis of all studies	T vs. C	21(6692/8266)	0.92(0.85-1.01)	0.068	R 59.90%
	TT+CT vs. CC	21(6692/8266)	0.91(0.85-0.98)	0.011	F 26.30%
	TT vs. CC	21(6692/8266)	0.84(0.70-1.00)	0.055	R 59.90%
	TT vs. CC+CT	21(6692/8266)	0.85(0.73-1.00)	0.053	R 58.30%
Korea	T vs. C	6(3381/3542)	0.88(0.78-0.99)	0.029	R 50.00%
	TT+CT vs. CC	6(3381/3542)	0.91(0.82-1.00)	0.054	F 0.00%
	TT vs. CC	6(3381/3542)	0.77(0.66-0.88)	<0.001	F 48.90%
	TT vs. CC+CT	6(3381/3542)	0.76(0.60-0.97)	0.025	R 58.80%
Japan	T vs. C	4(1189/2012)	0.88(0.79-0.97)	0.015	F 18.00%
	TT+CT vs. CC	4(1189/2012)	0.88(0.76-1.03)	0.107	F 29.00%
	TT vs. CC	4(1189/2012)	0.74(0.59-0.92)	0.007	F 5.30%
	TT vs. CC+CT	4(1189/2012)	0.77(0.63-0.95)	0.014	F 0.00%
China	T vs. C	10(1992/2582)	1.01(0.86-1.20)	0.863	R 70.80%
	TT+CT vs. CC	10(1992/2582)	0.97(0.85-1.09)	0.575	F 48.20%
	TT vs. CC	10(1992/2582)	1.01(0.71-1.44)	0.964	R 71.00%
	TT vs. CC+CT	10(1992/2582)	1.00(0.74-1.35)	0.985	R 67.00%
China (Low plasma folate region)	T vs. C	6(1310/1495)	1.11(1.00-1.24)	0.06	F 49.90%
	TT+CT vs. CC	6(1310/1495)	1.10(0.94-1.29)	0.248	F 19.60%
	TT vs. CC	6(1310/1495)	1.25(1.00-1.56)	0.055	F 48.30%
	TT vs. CC+CT	6(1310/1495)	1.22(1.00-1.49)	0.047	F 36.60%
China (High plasma folate region)	T vs. C	4(682/1087)	0.77(0.67-0.89)	<0.001	F 39.10%
	TT+CT vs. CC	4(682/1087)	0.79(0.65-0.96)	0.019	F 36.30%
	TT vs. CC	4(682/1087)	0.55(0.40-0.74)	<0.001	F 18.20%
	TT vs. CC+CT	4(682/1087)	0.59(0.44-0.78)	<0.001	F 0.00%

*M, model of meta-analysis; R, random-effects model; F, Fixed-effects model

Kim et al., 2011; Zhu et al., 2011; Kim et al., 2012). The detailed characteristics of these studies are summarized in Table 1. We further classified the studies from China into two groups according to the plasma folate level (Hao et al., 2002). There were 17 studies with high quality and 4 ones with low quality (Table 2).

Meta-analysis results

The main results of this meta-analysis were shown in Table 2. The combined results based on all studies showed

that the T variant of MTHFR C677T polymorphism contributed to decreased CRC risk under the dominant genetic comparison model (OR =0.91, 95%CI=0.85-0.98, P=0.011). However, there was no obvious association under the other three genetic models (Table 3). Subgroup analyses by country further showed that the T variant of MTHFR C677T polymorphism contributed to decreased CRC risk in Japan and Korea, but not in China (Table 3). The sensitivity analysis by omission of studies in

Table 4. Summary of Pooled Odds Ratios (OR) with Confidence Interval (CI) in the Meta-analysis of 17 HWE Studies

Comparison Model	Studies (cases / controls)	Odds Ratio		M*	I ²	
		OR[95%CI]	P _{OR}			
Analysis of all 17 studies	T vs. C	17(5325/6362)	0.94(0.85- 1.05)	0.263	R	65.70%
	TT+CT vs. CC	17(5325/6362)	0.92(0.85- 0.99)	0.026	F	40.90%
	TT vs. CC	17(5325/6362)	0.88(0.71-1.10)	0.263	R	64.80%
	TT vs. CC+CT	17(5325/6362)	0.90(0.75-1.09)	0.269	R	59.50%
China	T vs. C	9(1867/2242)	1.04(0.86- 1.25)	0.688	R	73.20%
	TT+CT vs. CC	9(1867/2242)	1.02(0.84-1.24)	0.85	R	53.90%
	TT vs. CC	9(1867/2242)	1.07(0.73-1.57)	0.742	R	72.80%
	TT vs. CC+CT	9(1867/2242)	1.05(0.76-1.46)	0.745	R	67.60%
Korea	T vs. C	3(2139/1978)	0.83(0.62-1.12)	0.226	R	73.70%
	TT+CT vs. CC	3(2139/1978)	0.90(0.79-1.03)	0.13	F	38.30%
	TT vs. CC	3(2139/1978)	0.69(0.38-1.24)	0.212	R	71.20%
	TT vs. CC+CT	3(2139/1978)	0.72(0.43-1.21)	0.213	R	69.50%
Japan	T vs. C	4(1189/2012)	0.88(0.79-0.97)	0.015	F	18.00%
	TT+CT vs. CC	4(1189/2012)	0.88(0.76-1.03)	0.107	F	29.00%
	TT vs. CC	4(1189/2012)	0.74(0.59-0.92)	0.007	F	5.30%
	TT vs. CC+CT	4(1189/2012)	0.77(0.63-0.95)	0.014	F	0.00%

*M, model of meta-analysis; R, random-effects model; F, Fixed-effects model

which controls were found not to be in HWE showed that the significance of ORs didn't in the analysis of total studies and subgroup analyses of Japan and China, which suggested that the outcomes above were credible (Table 4). However, the significance of ORs in the sensitivity analysis of Korea changed (Table 4).

There was obvious heterogeneity in the subgroup analyses of China, and meta-regression showed that the plasma folate was the main source of heterogeneity in this meta-analysis. So we further performed subgroup analyses by the plasma folate. The outcomes showed that T variant of MTHFR C677T polymorphism was associated with decreased CRC risk in persons with high plasma folate while T variant of MTHFR C677T polymorphism was associated with increased CRC risk in persons with low plasma folate (Table 3).

Sensitivity analyses

A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data set on the pooled ORs, and most of the corresponding pooled ORs were not materially altered (data not shown).

Publication bias

Funnel plot and Egger's test were used to assess publication bias. The shape of the funnel plots was symmetrical, and the Egger test further provided evidence that there was no publication bias among the studies included. Thus, the publication bias was not obvious in this meta-analysis (data not shown).

Discussion

Previous studies investigating the association between MTHFR C677T polymorphism and CRC risk in Asian population have provided inconsistent results, and most of those studies involved no more than a few hundred CRC cases, which is too few to assess any genetic effects reliably. Meta-analysis has been recognized as an important tool to more precisely define the effect of

selected genetic polymorphisms on risk of disease and to identify potentially important sources of between-study heterogeneity. Previous meta-analysis conducted by Taioli E et al. included only 8 case-control studies in the analysis of Asian population, which was too little to confirm the association between MTHFR C677T polymorphism and CRC risk. Hence, to provide the most comprehensive assessment of the association between MTHFR C677T polymorphism and CRC risk in Asian population, we did an updated meta-analysis of all available studies. At last, we performed this meta-analysis by critically reviewing 21 individual case-control studies on MTHFR C677T polymorphism (a total of 6,692 cases and 8,266 controls). Compared with last meta-analysis conducted by Taioli E et al, we performed this updated meta-analysis on the association between MTHFR C677T polymorphism and CRC risk in Asian population by including at another 13 new case-control studies. Finally, we found that there was an obvious association of MTHFR 677T allele with decreased risk of colorectal cancer (OR = 0.91, 95%CI = 0.85-0.98, P = 0.011). Subgroup analyses by country further identified this association. Meta-regression showed that the dietary folate was the main source of heterogeneity in this meta-analysis.

Heterogeneity is a potential problem when interpreting the results of all meta-analyses, and finding of the sources of heterogeneity is one of the most important goals of meta-analysis (Higgins et al., 2003). In this study, we assessed the between-study heterogeneity by using the I² statistic to quantify the between-study heterogeneity (Higgins et al., 2003). Generally, the between-study heterogeneity was obvious in the subgroup analyses of China population. To study the source of between-study heterogeneity, meta-regression was also performed (Thompson et al., 2002), and meta-regression showed that the plasma folate was the main source of heterogeneity in this meta-analysis of China population. Hao L's study showed there existed significant difference in plasma folate concentrations in adults between varied geographic areas in China, and our meta-analysis suggested plasma

folate was the main source of heterogeneity in this meta-analysis. So we further performed subgroup analyses by the plasma folate in the subgroup analysis of China. The outcomes showed that T variant of MTHFR C677T polymorphism was associated with decreased CRC risk in persons with high plasma folate while T variant of MTHFR C677T polymorphism was associated with increased CRC risk in persons with low plasma folate (Table 3). Thus, the MTHFR 677T allele is also associated with risk of colorectal cancer in China population, and there is effect modification by population plasma folate on this association.

Possible limitations of this meta-analysis have to be considered in explaining the results. Firstly, publication bias may have occurred because only published researches were included in this study. Though Funnel plot was performed to access the publication bias in this meta-analysis and the outcome suggested that publication bias was not evident in this meta-analysis, but the publication bias in the present analysis was still not negligible. Secondly, misclassification bias was possible. For example, most studies could not exclude latent cancer cases in the control group. The controls in some studies were selected from non-cancer patients, while the controls in other several studies were just selected from asymptomatic individuals. Finally, gene-environmental interactions were not fully addressed in this meta-analysis for the lack of sufficient data. As we know, aside from genetic factor, smoking and some environmental risk factors are major risk factors for CRC; however we didn't perform subgroup analyses based on environmental exposure owing to the limited reported information on such associations in the included studies. Considering these limitations, our results should be interpreted with caution.

Despite of those limitations, this meta-analysis suggests that the MTHFR 677T allele is associated with lower risk of colorectal cancer in Asian population, and there is effect modification by population plasma folate on this association.

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The author(s) declare that they have no competing interests.

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