RESEARCH ARTICLE

Evaluation of the MTHFR C677T Polymorphism as a Risk Factor for Colorectal Cancer in Asian Populations

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Abstract

Background: Genetic and environmental factors play important roles in pathogenesis of digestive tract cancers like those in the esophagus, stomach and colorectum. Folate deficiency and methylenetetrahydrofolate reductase (MTHFR) as an important enzyme of folate and methionine metabolism are considered crucial for DNA synthesis and methylation. MTHFR variants may cause genomic hypomethylation, which may lead to the development of cancer, and MTHFR gene polymorphisms (especially C677T and A1298C) are known to influence predispositions for cancer development. Several case control association studies of MTHFR C677T polymorphisms and colorectal cancer (CRC) have been reported in different populations with contrasting results, possibly reflecting inadequate statistical power. Aim: The present meta-analysis was conducted to investigate the association between the C677T polymorphism and the risk of colorectal cancer. Materials and Methods: A literature search of the PubMed, Google Scholar, Springer link and Elsevier databases was carried out for potential relevant articles. Pooled odds ratio (OR) with corresponding 95 % confidence interval (95 % CI) was calculated to assess the association of MTHFR C677T with the susceptibility to CRC. Cochran's Q statistic and the inconsistency index (I^2) were used to check study heterogeneity. Egger's test and funnel plots were applied to assess publication bias. All statistical analyses were conducted by with MetaAnalyst and MIX version 1.7. Results: Thirty four case-control studies involving a total of 9,143 cases and 11,357 controls were retrieved according to the inclusion criteria. Overall, no significant association was found between the MTHFR C677T polymorphism and colorectal cancer in Asian populations (for T *vs.* C: OR=1.03; 95% CI= 0.92-1.5; p= 0.64; for TT *vs* CC: OR=0.88; 95%CI= 0.74-1.04; p= 0.04; for CT *vs.* CC: OR = 1.02; 95%CI= 0.93-1.12; p=0.59; for TT+ CT *vs.* CC: OR=1.07; 95%CI= 0.94-1.22; p=0.87). Conclusions: Evidence from the current meta-analysis indicated that the C677T polymorphism is not associated with CRC risk in Asian populations. Further investigations are needed to offer better insight into any role of this polymorphism in colorectal carcinogenesis.

Keywords: Colorectal cancer - MTHFR - C677T - meta-analysis - homocysteine - folate

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Introduction

Colorectal cancer (CRC) is the second most common cancer worldwide and 60% cases occur in developed regions. The incidence varies considerably among different ethnicities (Parkin et al., 1999; Ferlay et al., 2008; Center et al., 2009; Jemal et al., 2009; Yousef et al., 2014). Although incidence rates are lower in Asian than Caucasian populations (Jang et al., 2013). Fifty percentage of the people diagnosed with colorectal cancer will die of the disease (Boyle and Ferlay, 2005; Li et al., 2011). CRC is a multifactorial disease with involvement of both genetic and environmental factors. Epidemiological studies have indicated that diets plays an important role in colorectal malignancy, high intake of alcohol and red meat and low intake of folate i.e green vegetables, fruits and dietary fibres are increased the risk of CRC (Russo et al., 1998; Bedine, 1999; Boutron-Ruault et al., 2001; Giovannucci, 2001; Giovannucci et al., 1995). High folate intake has protective effect against CRC (Giovannucci et al., 1995; Wang et al., 2006). Folate metabolism is regulated by several enzymes and out of which methylenetetrahydrofolate reductase (MTHFR) is the critical enzyme and genetic polymorphism of MTHFR enzyme is considered as a potential marker that may influence the risk of CRC.

MTHFR enzyme is involved in DNA synthesis, repair and methylation (Sameer et al., 2011). It is responsible for determining whether reduced folates are directed towards DNA methylation pathways or DNA synthesis (Ryan and Weir, 2001). The MTHFR locus has been mapped to chromosome 1p36.3 in humans (Goyette et al., 1994). The most well-studied polymorphism in MTHFR gene is the replacement of the nucleotide thymine by cytosine at position 677 (Ala222Val) (Frosst et al., 1995). This variant, which is relatively common in many populations

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worldwide, produces a form of methylenetetrahydrofolate reductase that reduces activity at higher temperatures, leading to lower levels of circulating folate (5-methyl-THF), an accumulation of 5, 10-methylene-THF, and increased plasma homocystein levels (Ma et al., 1997; Yan et al., 2012). The frequency of T allele varies in different ethnic population. According to HapMap database, the highest frequency i.e 0.48 is reported from Chinese Han population and T allele frequency in Europeans, Africans, Japanese and Indians is reported as 0.31, 0.08 to 0.12, 0.38 and 0.16 respectively (http:// hapmap.ncbi.nlm.nih.gov). Previous studies have suggested that T allele frequency can be higher in regions where food and vitamins are sufficient, as higher homocysteine and low-folate levels are compensated (Kennedy et al., 2012). MTHFR C677T had been suggested as a possible risk factor for congenital anomalies, heart disease, stroke, diabetes, psychiatric disorders and certain types of cancer (Fu et al., 2013; Rai et al., 2014; Rai 2014a, b).

DNA strand breaks, impaired DNA methylation and repair have been associated with folate deficiency and CRC (Duthie, 1999; Ames, 2001; Fenech, 2001; Kim, 2003). Several studies from around the globe have indicated that the common polymorphisms in the MTHFR gene might play a critical role in increasing susceptibility to CRC (Park et al., 1999; Yin et al., 2004). However, some other studies suggested that the MTHFR C677T polymorphisms were not associated with susceptibility to CRC (Chen et al., 1996; Ma et al., 1997; Slattery et al., 1999; Sachse et al., 2002; Shannon et al., 2002). In view of the conflicting results from previous studies, present meta-analysis of all available data was performed to evaluate the association between MTHFR C677T polymorphisms and susceptibility to CRC.

Materials and Methods

Literature search strategy and inclusion criteria:

Meta-analysis was performed according to MOOSE guidelines (Stroup et al., 2000). A literature search of the PubMed, Google Scholar, Elsevier and Springer Link databases was conducted to identify articles published up to July, 2014, with following keywords: using the following terms: (''genetic polymorphism'' or ''polymorphism'' or ''SNP'' or ''single nucleotide polymorphism'' or ''gene mutation'' or ''genetic variants'') and (''Colorectal cancer'' or ''colon cancer'' or ''rectal cancer'') and (''MTHFR'' or ''methylenetetrahydrofolate reductase'' or ''C677T''). Bibliographies of review articles were manually searched to find additional eligible studies. If more than one study by the same author using the same case series was published, either the studies with the largest sample size or the most recently published study was included. Included studies had to fit the following criteria:(1) sufficient data regarding allele frequency; (2) an association analysis between the MTHFR C677T polymorphism and CRC risk; and (3) independent case-control studies.

Data extraction

The following information was extracted from the each identified studies: the first author family name, year of publication, sample size, country name, genotyping method, the numbers of patients and controls, MTHFR C677T genotypes information and frequencies of alleles in all study. For data not provided in the main text, the relevant information was obtained by contacting corresponding authors.

Statistical analysis

The strength of the association between MTHFR C677T polymorphisms and colorectal risk was measured by using crude odds ratio (OR) with 95% confidence interval (CI). The pooled ORs were estimated in following genetic models: allele contrast (T *vs*. C), codominant model (CT *vs*. CC), homozygote model (TT *vs*. CC), dominant model (TT+CT *vs*. CC), and recessive model (TT *vs*. CT+CC). Heterogeneity among studies was examined with the χ^2 test-based Q statistics and $P < 0.05$ was considered significant. Heterogeneity was quantified with the I^2 metric, which is independent of the number of studies in the meta-analysis (Zintzaras and Lau, 2008). I^2 takes values between 0% and 100%, with higher values indicating a greater degree of heterogeneity. Fixed-effects summary ORs were calculated using the Mantel-Haenszel method (Mantel and Haenszel, 1959), and the DerSimonian and Laird method was used to calculate random-effects summary ORs (DerSimonian and Laird, 1986).

Egger's test and an inverted funnel plot was used to assess publication bias (Begg and Mazumdar, 1994; Egger et al., 1997). HWE was checked in the control group of the eligible studies by the chi-square test ($p \leq 0.05$). Sensitivity analysis was performed including studies that deviated from HWE and with small sample size. Methodological quality was assessed according to the Newcastle-Ottawa Scale (NOS) criteria (Stang, 2010). The NOS criteria includes three aspects: (1) subject selection: 0, 4 points; (2) comparability of subject: 0, 2 points; (3) clinical outcome: 0, 3 points. NOS scores range from 0 to 9 with a score >7 indicating good quality (Yan et al., 2013). All statistical analysis was undertaken using the program MetaAnalyst and MIX version 1.7 (Bax et al., 2006). All P values were two-sided.

Results

Eligible studies:

A flow chart summarizing the process of study selection is shown in Figure 1. Initially, the highly sensitive search strategy of Pubmed, Google Scholar, Elsevier and Springer Link databases, 383 articles were retrieved. After screening the titles and abstracts of all retrieved articles, 189 articles were excluded. Then full texts were reviewed and 85 articles were further excluded and remaining 109 articles were further reviewed. Another 20 studies were excluded because 16 studies reported only cases and 4 studies were meta-analysis. 55 articles from remaining 89 articles were again excluded because studied population was not Asian. Based on the inclusion and exclusion criteria, finally, thirty four studies met the inclusion criteria and were included in the present met-

analysis (Park et al., 1999; Le Marchand et al., 2002; Matsuo et al., 2002; Huang et al., 2003; Kim et al., 2004; Yin et al., 2004; Jiang et al., 2005; Matsuo et al., 2005; Miao et al., 2005; Otani et al., 2005; Wang et al., 2006; Chang et al., 2007; Jin et al., 2007; Zeybek et al., 2007; Cao et al., 2008; Haghighi et al., 2008; Mokarram et al., 2008; Zhang et al., 2008; El-Awady et al., 2009; Chandy et al., 2010; Cui et al., 2010; Naghibalhossaini et al., 2010; Promthet et al., 2010; Yang et al., 2010; Zhu et al., 2010; Kang et al., 2011; Kim et al., 2011; Li et al., 2011; Prasad and Wilkhoo, 2011; Sameer et al., 2011; Zhu et al., 2011; Yin et al., 2011; Kim et al., 2012; Ozen et al., 2014).

Characteristic of included studies

The main characteristics of the included studies were shown in Table 1. A total of 20, 500 subjects were involved in this meta-analysis, including 9, 143 CRC patients and 11, 357 healthy controls. The publication years of the involved studies ranged from 1999 to 2014. Sample size ranged from 35 to 1, 829 in cases and 53 to 1700 in controls. Most reports presented demographic information regarding cases and controls. Genotyping was performed consistently across studies with polymerase chain reaction (PCR). In controls genotype percentage of CC, CT and TT were 40.45%, 43.30% and 16.24% respectively. In cases genotype percentage of CC, CT and TT were 40.59%, 45.66% and 13.75% respectively. Except four studies (Haghighi et al., 2008; Kang et al., 2011; Sameer et al., 2011; Kim et al., 2012), distribution of genotypes in the controls of thirty studies was consistent with Hardy-Weinberg equilibrium. OR of fourteen studies (Matsuo et al., 2002; Kim et al., 2004; Miao et al., 2005; Wanget al., 2006; Chang et al., 2007; Cao et al., 2008; El-Awady et al., 2009; Chandy et al., 2010; Naghibalhossaini et al., 2010; Zhu et al., 2010; Prasad and Wilkhoo, 2011; Sameer et al., 2011; Zhu et al., 2011; Ozen et al., 2014) was above one and other twenty studies did not show any association between MTHFR C677T polymorphism and CRC. All these thirty four studies were performed in

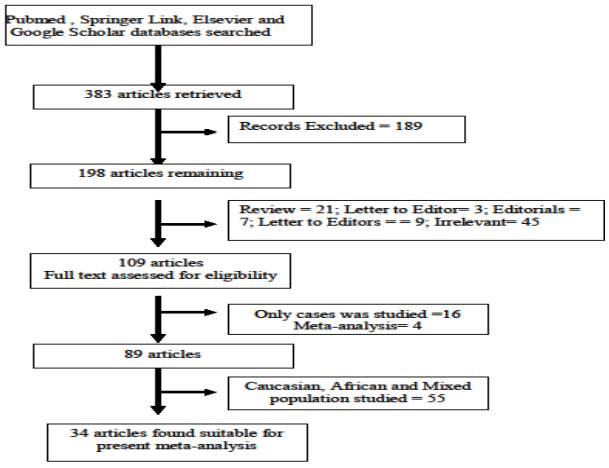

Figure 1. Flow Chart Shows Study Selection Procedure. Thirty four case control studies were included in this meta-analysis

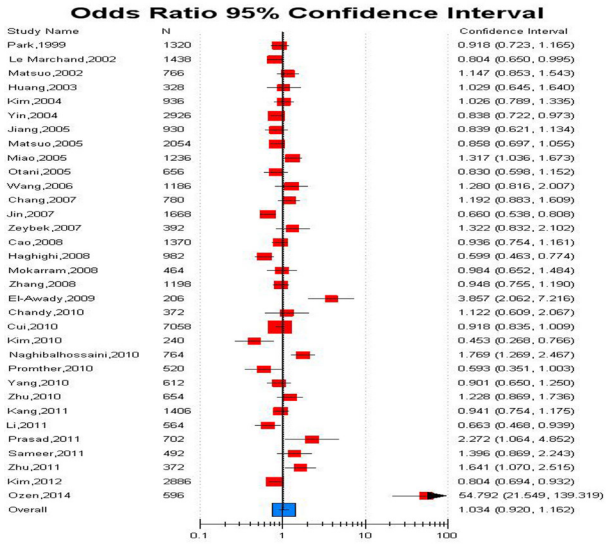

Figure 2. Forest Plots for the Association between MTHFR C677T Polymorphism and Colorectal Cancer for Allele Contrast Model (T vs C) with Fixed Effect Model in Asian Population

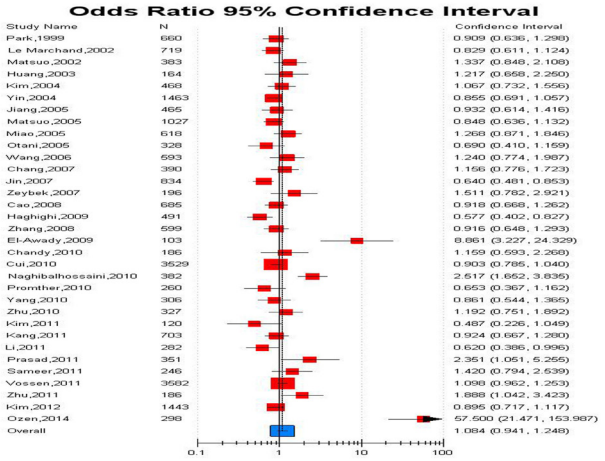

Figure 3. Forest Plots for the Association between MTHFR C677T Polymorphism and Colorectal Cancer for Dominant Model (TT+CT vs CC) with Fixed Effect Model in Asian Population

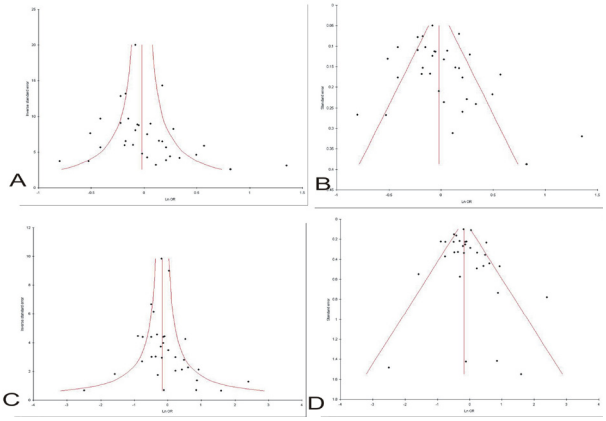

Figure 4. Funnel Plots. A. precision versus OR for additive model (T vs. C), B. standard error versus OR for additive model (T vs. C),C. precision versus OR for TT+CT vs CC, D. standard error versus OR for TT+CT vs CC.

different countries -China (Huang et al., 2003; Jiang et al., 2005; Miao et al., 2005; Chang et al., 2007; Jin et al., 2007; Cao et al., 2008; Zhang et al., 2008; Yang et al., 2010; Zhu et al., 2010; Li et al., 2011; Zhu et al., 2011), Egypt (El-Awady et al., 2009), India (Wang et al., 2006; Chandy et al., 2010; Prasad and Wilkhoo, 2011; Sameer et al., 2011), Iran (Haghighi et al., 2008; Mokarram et al., 2008; Naghibalhossaini et al., 2010), Korea (Park et a., 1999; Kim et al., 2004; Cui et al., 2010; Kim et al., 2011; Kang et al., 2011; Kim et al., 2012), Japan (Le Marchand et al., 2002; Matsuo et al., 2002; 2005; Yin et al., 2004; Otani et al., 2005), Thailand (Promther et al., 2010), Turkey (Zeybek et al., 2007; Ozen et al., 2014).

Meta-analysis

The association between MTHFR C677T and CRC risk in allele contrast, dominant, homozygote, co-dominant and recessive models was shown in Table 2. Meta-analysis with allele contrast (T vs C) did not show association with both fixed effect (OR_{TvsC}=0.94; 95%CI=0.90-0.98; p=0.001; I^2=74.54%; $P_{heterogeneity}$<0.0001; P_{Pb}=0.12) and random effect model (OR_{TvsC}=1.03; 95% CI=0.92-1.5; p=0.64) (Table 2, Figure 2).

Increased risk of colorectal cancer was not observed among mutant homozygote variants (TTvsCC; homozygote model) using both fixed (OR_{TTvsCC}=0.81; 95%CI=0.75-0.88; p <0.0001; I^2=61.88.84%; $P_{heterogeneity}$ <0.0001; P_{Pb}=0.27) and random (OR_{TTvsCC}=0.88; 95%CI=

Table 1. Characteristics of Thirty four Studies Included in the Present Meta-analysis

Study	Country	Case No.	Control No.	P value of HWE
Park et al., 1999	Korea	200	460	0.05
Le Marchand et al., 2002	Japan	322	397	0.22
Matsuo et al., 2002	Japan	142	241	0.3
Yin et al., 2002	Japan	685	778	0.52
Huang et al., 2003	China	82	82	0.58
Kim et al., 2004	Korea	243	225	0.77
Yin et al., 2004	Japan	685	778	0.52
Jiang et al., 2005	China	125	340	0.33
Matsuo et al., 2005	Japan	256	771	0.09
Miao et al., 2005	China	198	420	0.52
Otani et al., 2005	Japan	106	222	0.67
Wang et al., 2006	Indian	302	291	0.26
Chang et al., 2007	China	195	195	0.46
Jin et al., 2007	China	334	500	0.34
Zeybek et al., 2007	Turkey	52	144	0.8
Cao et al., 2008	China	315	370	0.82
Haghighi et al., 2008	Iran	234	257	0
Mokarram et al., 2008	Iran	151	81	0.31
Zhang et al., 2008	China	300	299	0.26
El-Awady et al., 2009	Egypt	35	68	0.41
Chandy et al., 2010	India	100	86	0.78
Cui et al., 2010	Korea	1829	1700	0.13
Kim et al., 2011	Korea	67	53	0.13
Naghibalhossaini et al., 2010	Iran	151	231	0.16
Promther et al., 2010	Thailand	130	130	0.24
Yang et al., 2010	China	141	165	0.51
Zhu et al., 2010	China	216	111	0.23
Kang et al., 2011	Korea	255	448	0.04
Li et al., 2011	China	137	145	0.33
Prasad and Wilkhoo,2011	India	110	241	0.07
Sameer et al., 2011	India	86	160	0
Zhu et al., 2011	China	86	100	0.74
Kim et al., 2012	Korea	787	656	0.003
Ozen et al., 2014	Turkey	86	212	0.86

Table 2. Summary estimates for the odds ratio (OR) of MTHFR C677T in various allele/genotype contrasts, p value of heterogeneity test (Q test), I^2 metric and p-value of Egger test

OR	Fixed effect (OR (95% CI), p)	Random effect (OR (95% CI), p)	Heterogeneity p-value (Q test)	I^2 (%)	Publication Bias (p of Egger's test)
Allele Contrast (T vs C)	0.94(0.90-0.98), 0.001	1.03(0.92-1.5), 0.64	<0.0001	74.54	0.12
Co-dominant (CT vs CC)	1.01(0.95-1.1), 0.79	1.02(0.93-1.12), 0.59	0.0003	52.04	0.17
Homozygoote (TT vs CC)	0.81(0.75-0.88), <0.0001	0.88(0.74- 1.04), 0.04	<0.0001	61.88	0.27
Dominant (TT+CT vs CC)	0.96(0.90-1.01), 0.16	1.07(0.94-1.22), 0.87	<0.001	65.6	0.17
Recessive (TT vs CT+CC)	0.82(0.76-0.88), <0.0001	0.84(0.74-0.96), 0.01	0.0002	53.1	0.39

0.74-1.04; p=0.04) effect models and high statistical heterogeneity was also found. Association of mutant heterozygous genotype (CT *vs*.CC; co-dominant model) was not observed significant with both fixed (OR_{CTvsCC}= 1.01; 95%CI=0.95-1.1; p= 0.79; I^2=52.04%; $P_{heterogeneity}$= 0.0003; PPb=0.17) and random (OR_{CTvsCC}= 1.02; 95%CI= 0.93-1.12; p=0.59) effect models. Similarly combined mutant genotypes (TT+CT *vs*. CC; dominant model) did not show positive association with colorectal cancer using both fixed ($OR_{TT+CTvsCC}$=0.96; 95%CI=0.90-1.01; p=0.16; I^2=65.6%; $P_{heterogeneity}$<0.0001; P_{Pb}=0.17) and random ($OR_{TT+CTvsCC}$=1.07; 95%CI=0.94-1.22; p=0.87) effect models (Table 2; Figure 3).

Sensitivity analysis

Sensitivity analyses by sequential omission of individual studies or studies with low quality (studies departed from HWE or with small sample size) did not alter the overall pooled ORs. The genotype distribution of the control population in four studies (Haghighi et al., 2008; Kang et al., 2011; Sameer et al., 2011; Kim et al., 2012) was not in HWE. Overall, the results from sensitivity analysis after exclusion of these four studies were similar to those from nonsensitivity analysis, and MTHFR C677T allele or TT genotype was not associated with CRC susceptibility(OR_{TvsC}=0.96; 95%CI=0.91-1.00; p=0.008; I^2=83.6%; $P_{heterogeneity}$<0.0001).

Sensitivity analysis for the relationship between the MTHFR C677T gene polymorphism and CRC risk according to sample size of case (<100) was also performed. The results of exclusion of seven studies with samples size less than 100 (Huang et al., 2003, n=82; Zeybek et al., 2007, n=52; El-Awady et al., 2009, n=35; kim et al., 2010, n=67; Sameer et al., 2011, n=86; Zhu et al., 2011, n=86; Ozen et al., 2014, n=86) were similar as the non-sensitivity analysis, and T allele was not associated with CRC susceptibility (OR_{TvsC}= 0.93; 95%CI=0.89-0.97; p= 0.001; I^2=71.04%; $P_{heterogeneity}$ <0.0001).

Bias diagnosis

Begg funnel plot and Egger test were performed to assess publication bias. Egger test was used to provide statistical evidence for funnel plot symmetry. The Funnel plots' shape of all contrasts did not reveal obvious evidence of asymmetry (Figure 4A-D), and all the P values of Egger's test were more than 0.05, providing statistical evidence for the funnel plots' symmetry (Table 2).

Discussion

In present study, the possible association between the C677T polymorphisms of MTHFR gene and CRC was investigated with a meta-analysis. The results showed that MTHFR C677T polymorphism is not associated with CRC. Colorectal cancer is an important clinical problem which has been well-studied but its mechanism is still relatively unclear. DNA methylation is an important epigenetic process for transcriptional control of human genome including those genes involved in cancer initiation and progression. Clinical studies have suggested that biological explanation to the protective effect of some nutrients could be linked with the DNA methylation (Galvan-Portillo et al., 2009). The metabolism of folate is significant for the maintenance of genome integrity due to its role in DNA synthesis, repair, and methylation (Fowler, 2005). Defects in folate metabolism can arise from a poor dietary intake of folates and other nutrients or may be determined genetically as a result of the combined influence of many low-penetration mutations or single nucleotide polymorphisms (SNPs). These genetic polymorphisms could modify the activity, stability, or level of the corresponding enzymes and thereby impair folate absorption or disturb the balance between folate derivatives (Weiwei et al., 2014).

Several large-scale meta-analyses combining data from multiple studies have been published investigating the association between MTHFR C677T polymorphism and various cancers such as gastric, lung cancer, breast cancer, cervical cancer, and liver cancer (Zintzaras, 2006; Bai et al., 2009; Jin et al., 2009; Ding et al., 2012; Mei et al., 2012; Niu et al., 2012; Tu et al., 2012; Zhuo et al., 2012; Wen et al., 2013; You et al., 2013; Rai, 2014, a, b). Some reports demonstrated association between MTHFR C677T polymorphism and cancer (You et al., 2013; Rai, 2014a), whereas others reported contradictory results i.e no association (Ding et al., 2012; Niu et al., 2012). These inconsistent and confusing conclusions can be attributed to several factors like *(i)* different selection criteria and

selection bias might account for the diversity of the results, *(ii)* folate metabolic pathway is complex pathway and MTHFR is only one of many enzymes involved in the pathway, *(iii)* the studies with small sample size were included in the meta-analyses and these studies have lower statistical power than those with large sample size and *(iv)* finally different mechanisms of carcinogenesis of different cancers might due to gene-variant associations vary in different kinds of diseases (Qin et al., 2014). This contradiction suggests that the effect of the C677T polymorphism of MTHFR on the susceptibility to cancer may vary in different cancer type in different populations.

Heterogeneity is a critical issue in any meta-analysis, and an important aim of a meta-analysis is to determine the source and causes of heterogeneity. Evidence of between-study heterogeneities was observed in allelic contrast, co-dominant, homozygote, dominant and recessive models, therefore the random effect models were adopted and sensitivity analyses were done (Zhang et al., 2014). However, author failed to find the source of heterogeneity by conducting sensitivity analyses.

The present meta-analysis on CRC and MTHFR C677T association is more reliable than previous meta-analysis studies because it included the latest published articles and a larger sample size. However it has some limitations also. First, the results were based on unadjusted ORs, while a more precise evaluation should be adjusted by potentially confounding factors, including age, gender, body mass index, smoking status, drink abuse, and environmental factors. Second, the controls were not uniformly defined. Some studies used population-based controls, while others used hospital based controls. Third, single gene of folate pathway was considered in present meta-analysis. Finally, the effect of gene-gene and gene-environment interactions was not considered.

In summary, present meta-analysis indicated that C677T polymorphism in the MTHFR gene may not be associated with CRC susceptibility in Asians. Well-designed prospective studies with large sample size should be conducted to validate findings of the present meta-analysis..

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