Research Article

Methylenetetrahydrofolate reductase C677T polymorphism and colorectal cancer susceptibility: a meta-analysis

Lingyan Xu1,2,*, Zhiqiang Qin2,*, Feng Wang3,*, Shuhui Si4, Lele Li1, Peinan Lin1, Xiao Han1, Xiaomin Cai1, Haiwei Yang2 and Yanhong Gu1

1Department of Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China; 2State Key Laboratory of Reproductive Medicine, Department of Urology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China; 3Department of Radiation Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China; 4Research Division of Clinical Pharmacology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

Correspondence: Yanhong Gu (guyhphd@163.com) or Haiwei Yang (haiweiyang@njmu.edu.cn)

The association between methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and colorectal cancer (CRC) susceptibility has been researched in numerous studies. However, the results of these studies were controversial. Therefore, the objective of this meta-analysis was to offer a more convincing conclusion about such association with more included studies. Eligible studies published till May 1, 2017 were searched from PubMed, Embase, Web of Science, and CNKI database about such association. Pooled odds ratios (ORs) together with 95% confidence intervals (CIs) were calculated to evaluate such association. And the Begg’s funnel plot and Egger’s test were applied to assess the publication bias. This meta-analysis contained 37049 cases and 52444 controls from 87 publications with 91 eligible case-control studies. Because of lack of data for a particular genotype in several studies, all the included studies were analysed barely in the dominant model. Originally, there was no association between MTHFR C677T polymorphism and CRC susceptibility (OR=0.99, 95% CI=0.94–1.05). After excluding 13 studies according to their heterogeneity and publication bias, rs1801133 polymorphism was found to reduce the risks of CRC significantly (OR=0.96, 95% CI=0.94–0.99). In the subgroup analysis of ethnicity, there was a significant association in Asians (OR=0.94, 95% CI=0.89–1.00). Furthermore, when stratified by the source of controls and genotyping methods, the positive results were observed in population-based control group (OR=0.97, 95% CI=0.93–1.00) and PCR-restriction fragment length polymorphism (PCR-RFLP) method (OR=0.95, 95% CI=0.91–0.99. The results of the meta-analysis suggested that MTHFR C677T polymorphism was associated with CRC susceptibility, especially in Asian population.

Introduction

Colorectal cancer (CRC) is a critical public health problem, which is the third most commonly diagnosed cancer and the third common cause of cancer deaths in both males and females. There were 134,490 new CRC cases and 49,190 mortalities by estimation in the United States in 2016 [1]. The colorectal carcinogenesis is a complex multistep process (a benign adenomatous polyp – an advanced adenoma with high-grade dysplasia – an invasive cancer) with altered expression of oncogenes, tumor suppressor genes and DNA repair genes [2]. However, the etiology of CRC is still unclear. It is known to all that CRC is a multifactorial and multigenic disease, and is influenced by environment conditions, diet habits, genetic
mutations, and Escherichia coli infection [3,4]. With increasing numbers of studies, more gene polymorphisms were found to contribute to CRC [5]. These single nucleotide polymorphisms (SNPs) can be used as markers for improving cancer diagnosis and determination of treatment plans [6].

As a key enzyme and an important regulator for the metabolism of folate/vitamin B<sub>9</sub>, methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate [7]. Simultaneously, the 5-methyltetrahydrofolate is the main circulatory form of folate in the body and provides a methyl group to convert the amino acid homocysteine into methionine, which is the precursor of S-adenosylmethionine (SAM). SAM is the major methyl donor in the cell and takes part in DNA methylation [8]. Therefore, MTHFR not only plays a role in making proteins and other important compounds, but also is an important factor in DNA methylation, synthesis, and repair [9]. The enzyme is encoded by the MTHFR gene located on the short arm of chromosome 1-1p36.3 [10]. Previously, several mutations of MTHFR gene have been found and MTHFR C677T (rs1801133) is the most common type amongst them. MTHFR C677T represents an alanine-to-valine substitution at nucleotide position 677 in exon 4 resulting in thermolability and concurrent decreased activity of the enzyme [11,12]. MTHFR gene mutations lead to MTHFR enzyme deficiency, low plasma folate levels, hyperhomocysteinemia [13,14] and certain diseases such as cardiovascular disease, pregnancy complications, neural defect, and several cancers including CRC [15-21]. With a growing number of studies conducted to explore such association, we hypothesized that rs1801133 was likely to relate to colorectal carcinogenesis.

Many researchers have carried out a large number of studies to examine the potential association between MTHFR C677T polymorphism and CRC susceptibility. But, the results are still inconclusive so far. Thus, the aim of this meta-analysis including all available case–control studies was to investigate a more reliable association.

**Materials and methods**

We searched several databases including PubMed, Embase, Web of Science, and CNKI database for published studies about exploring the association between MTHFR C677T polymorphism and CRC susceptibility till May 1, 2017. The search strategy included listed key words: 'methyltetrahydrofolate reductase', 'MTHFR polymorphism', 'C677T', 'rs1801133', and 'risk or susceptibility' and 'colorectal or colon or rectal cancer'. Furthermore, we manually searched the reference lists of clinical trials and former meta-analyses for more relevant studies. When duplicate data appeared in different publications, this meta-analysis only adopted the most recent study or the study with the most complete information. The meta-analysis was on the basis of the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) [22]. The eligible studies needed to accord with the following inclusion criteria: (i) case–control studies; (ii) the language was not restricted to English; (iii) investigating the association between MTHFR C677T polymorphism and CRC susceptibility; (iv) offering enough raw data to calculate odds ratio (OR) with 95% confidence interval (CI). Additionally, exclusion criteria were as follows: (i) non–case–control studies; (ii) lack of sufficient data for calculating genotype frequency; (iii) case–control studies about examining the relationship between MTHFR C677T polymorphism and colorectal adenoma; (iv) duplicated publications.

**Data extraction**

In order to guarantee the accuracy of extracted information, two authors individually reviewed each publication and extracted useful data on the basis of the inclusion criteria listed above. When disagreements arose in the course of data extraction, discussion was carried out with other authors until the agreements were reached. The following information were extracted from each study to accomplish a standardized sheet: first author’s name, year of publication, ethnicity of population, source of controls (hospital based or population based), genotyping method, sample size of cases and controls, genotype frequency of rs1801133 in cases and controls, and the results of the Hardy–Weinberg equilibrium (HWE) test.

**Statistical analysis**

The relationship between MTHFR C677T polymorphism and CRC susceptibility was analyzed by using five models including the dominant model (CT + TT compared with CC), the recessive model (TT compared with CT + CC), the homozygous model (TT compared with CC), the heterozygous model (CT compared with CC), and the allele model (T compared with C). The goodness-of-fit χ<sup>2</sup> test was conducted to evaluate the HWE in control groups and P < 0.05 was regarded as significant disequilibrium [23]. Stratified analysis were performed by ethnicity, source of controls, and genotyping method. Besides, the pooled OR together with 95% CI were measured to bring out the strength of such association. The fixed effects model (Mantel–Haenszel method) and the random effects model (Dersimonian–Laird method) were selected to use based on heterogeneity in the meta-analysis. If there was no or little heterogeneity,
Figure 1. Flowchart of literature search and selection process

the fixed effects model was used; otherwise, the random effects model was used. Due to only particular genotypes extracted in several studies, the dominant model analysis were carried out for all the included studies [84]. Galbraith graph was performed to explore the impossible cause of heterogeneity [24]. A sensitivity analysis was conducted to assess the stability of the results. Begg’s funnel plot was performed for potential publication bias and Egger’s linear regression test was executed to assess funnel plot asymmetry statistically. If \( P < 0.05 \), publication bias existed [25]. All statistical data analyses were carried out by using Stata software (version 12.0, StataCorp LP, College Station, TX, U.S.A.).

**Results**

**Characteristics of the studies**

According to PRISMA-P, this meta-analysis contained 37049 cases and 52444 controls that were combined from 87 publications with 91 eligible case–control studies to examine the relationship between rs1801133 polymorphism and CRC risks [26-112]. The literature retrieval and selection process are shown in the flowchart in Figure 1. Detailed information of each study were listed in Table 1. The distribution of genotypes in controls was consistent with HWE except 15 studies [33-35,37,39,47,63,71,76,80,87,88,106,110,111]. In these studies, four ethnicities of population were included: Asian, Caucasian, African, and mixed ethnic group. Nine genotyping methods were applied: PCR-restriction fragment length polymorphism (PCR-RFLP), real-time PCR (RT-PCR), PCR-single
| Year | Surname (References) | Ethnicity | SOC | Genotyping | Case (n) | Control (n) | Case (%) | Control (%) | HWE |
|------|----------------------|-----------|-----|------------|---------|-------------|----------|-------------|-----|
| 2016 | Haerian [26]         | Asian     | HB  | Taqman     | 1123    | 1298        | 607      | 421         | 95  |
| 2015 | Kim [27]             | Asian     | PB  | PCR-RFLP   | 477     | 514         | 159      | 248         | 70  |
| 2014 | Rai [28]             | Asian     | PB  | PCR-RFLP   | 155     | 294         | 137      | 17          | 1   |
| 2014 | Ozen [29]            | Caucasian | PB  | RT-PCR     | 86      | 212         | 36       | 32          | 18  |
| 2013 | Ashmore [30]         | Caucasian | PB  | PCR-RFLP   | 625     | 603         | 241      | 309         | 75  |
| 2013 | Delgado-Plasencia    | Caucasian | HB  | PCR-RFLP   | 50      | 103         | 32       | 16          | 2   |
| 2013 | Yousef [32]          | Asian     | PB  | PCR-RFLP   | 86      | 100         | 29       | 42          | 15  |
| 2013 | Ashmore [33]         | Caucasian | PB  | Taqman     | 531     | 1004        | 317      | 307         | 42  |
| 2013 | Promthet [34]        | Caucasian | PB  | PCR-RFLP   | 112     | 242         | 93       | 18          | 1   |
| 2012 | Rai [35]             | Asian     | HB  | Taqman     | 787     | 656         | 265      | 393         | 129 |
| 2012 | Ozen [36]            | Caucasian | PB  | PCR-RFLP   | 370     | 370         | 124      | 167         | 79  |
| 2011 | Yousef [37]          | Asian     | PB  | PCR-RFLP   | 1762    | 1811        | 737      | 393         | 129 |
| 2011 | Delgado-Plasencia    | Caucasian | HB  | PCR-RFLP   | 255     | 448         | 87       | 134         | 34  |
| 2011 | Ashmore [38]         | Caucasian | PB  | Taqman     | 67      | 53          | 30       | 30          | 7   |
| 2011 | Promthet [39]        | Caucasian | PB  | PCR-RFLP   | 666     | 1376        | 317      | 307         | 42  |
| 2011 | Ashmore [40]         | Caucasian | HB  | PCR-RFLP   | 87      | 53          | 30       | 30          | 7   |
| 2011 | Prasad [41]          | Asian     | PB  | PCR-RFLP   | 110     | 241         | 97       | 12          | 1   |
| 2011 | Li [42]              | Asian     | PB  | PCR-RFLP   | 137     | 145         | 68       | 54          | 15  |
| 2011 | Jokic [43]           | Caucasian | PB  | Taqman     | 300     | 300         | 139      | 130         | 31  |
| 2011 | Guimarães(a) [44]    | Caucasian | PB  | PCR-RFLP   | 101     | 188         | 42       | 44          | 15  |
| 2011 | Guimarães(b) [45]    | African   | PB  | PCR-RFLP   | 12      | 188         | 6        | 6           | 0   |
| 2010 | Komlosi [46]         | Caucasian | PB  | PCR-RFLP   | 951     | 939         | 398      | 427         | 126 |
| 2010 | Karpinski [47]       | Caucasian | HB  | MSP        | 186     | 140         | 74       | 97          | 15  |
| 2010 | Cui [48]             | Asian     | PB  | PCR-RFLP   | 1829    | 1700        | 622      | 923         | 284 |
| 2010 | Eussen [49]          | Caucasian | PB  | MALDI-TOF-MS | 1329   | 2366        | 567      | 608         | 154 |
| 2010 | Prasad [50]          | Asian     | PB  | Sequenom   | 100     | 86          | 74       | 25          | 1   |
| 2010 | Naghibalhossaini     | Asian     | PB  | PCR-RFLP   | 151     | 231         | 64       | 80          | 7   |
| 2010 | Promthet [51]        | Caucasian | PB  | PCR-RFLP   | 130     | 130         | 104      | 26          | 0   |
| 2010 | Wang [52]            | Asian     | PB  | Sequenom   | 141     | 165         | 58       | 61          | 22  |
| 2010 | Fernández-Peralta    | Caucasian | HB  | PCR-RFLP   | 143     | 103         | 89       | 52          | 2   |
| 2010 | Zhu [53]             | Asian     | PB  | PCR-RFLP   | 216     | 111         | 88       | 102         | 26  |
| 2009 | Vogel [54]           | Caucasian | PB  | RT-PCR     | 689     | 1793        | 318      | 320         | 51  |
| 2009 | Iacopetta [55]       | Mixed     | PB  | PCR-RFLP   | 850     | 958         | 382      | 386         | 82  |
| 2009 | Arreola [56]         | Caucasian | PB  | PCR-RFLP   | 369     | 170         | 124      | 126         | 119 |
| 2009 | Reeves [57]          | Caucasian | HB  | Taqman     | 206     | 211         | 105      | 83          | 18  |
| 2009 | Awady [58]           | African   | PB  | PCR-RFLP   | 35      | 68          | 6        | 23          | 6   |
| 2009 | Derwing [59]         | Asian     | PB  | Taqman     | 544     | 299         | 273      | 216         | 55  |
| 2008 | Haghhighi [60]       | Asian     | HB  | PCR/RFLP   | 234     | 257         | 117      | 68          | 49  |
| 2008 | Sharp [61]           | Caucasian | PB  | PCR-RFLP   | 251     | 394         | 117      | 111         | 23  |
| 2008 | Kury [62]            | Caucasian | PB  | Taqman     | 1023    | 1121        | 435      | 452         | 136 |
| 2008 | Mokarram [63]        | Asian     | HB  | MSP        | 151     | 81          | 64       | 80          | 7   |
| 2008 | Cao [64]             | Asian     | PB  | PCR-RFLP   | 315     | 370         | 109      | 154         | 52  |
| 2008 | Theodoratou [65]     | Caucasian | PB  | MassARRAY  | 999     | 1010        | 447      | 441         | 111 |
| 2008 | Ekolf [66]           | Caucasian | PB  | Taqman     | 220     | 414         | 123      | 85          | 12  |
| 2008 | Zhang [67]           | Asian     | HB  | PCR-RFLP   | 300     | 299         | 97       | 136         | 67  |
| 2008 | Guerreiro [68]       | Caucasian | HB  | Taqman     | 196     | 200         | 94       | 76          | 26  |
| 2008 | Oisan [69]           | Caucasian | PB  | PCR-RFLP   | 69      | 67          | 38       | 25          | 6   |
| 2008 | Zeybek [70]          | Asian     | PB  | PCR-RFLP   | 52      | 144         | 18       | 27          | 7   |
| 2008 | Lima [71]            | Caucasian | PB  | PCR-RFLP   | 90      | 300         | 36       | 40          | 14  |

Continued over
Table 1 Characteristics of individual studies included in the meta-analysis (Continued)

| Year | Surname (References) | Ethnicity | SOC | Genotyping | Case (n) | Control (n) | Case | Control | HWE |
|------|-----------------------|-----------|-----|-------------|---------|-------------|------|---------|-----|
|      |                       |           |     |             |         |             |      |         |     |
| 2000 | Slattery [108]         | Caucasian | PB  | PCR-RFLP    | 232     | 232         | 164  | 164     | 3    |
|      |                       |           |     |             | 106     | 106         | 107  | 107     | 9    |
| 1999 | Slattery [109]         | Mixed     | PB  | PCR-RFLP    | 1467    | 1467        | 1821 | 1821    | 1    |
|      |                       |           |     |             | 673     | 673         | 655  | 655     | 0.96|
| 1999 | Park [110]             | Asian     | PB  | PCR-RFLP    | 200     | 200         | 460  | 460     | 6    |
|      |                       |           |     |             | 65      | 65          | 107  | 107     | 0.99|
| 1997 | Ma [111]               | Caucasian | PB  | PCR-RFLP    | 202     | 202         | 326  | 326     | 18   |
|      |                       |           |     |             | 92      | 92          | 92   | 92      | 0.9  |
| 1996 | Chen [112]             | Caucasian | PB  | PCR-RFLP    | 144     | 144         | 627  | 627     | 64   |
|      |                       |           |     |             | 67      | 67          | 64   | 64      | 0.95|

These 13 studies in bold were removed afterward because of its heterogeneity and publication bias. Abbreviations: HB: hospital-based control; PB, population-based control; SOC, source of control.

strand conformation polymorphism (PCR-SSCP), methylation-specific PCR (MS-PCR), mutagenically separated PCR (MSP), MALDI-TOF-MS, Taqman, MassARRAY, and Sequenom. Depending on different sources of control, population-based and hospital-based control groups were distinguished in all the included studies.

Results of quantitative synthesis

Initially, there was no association between MTHFR C677T polymorphism and CRC susceptibility in the dominant model (OR = 0.99, 95% CI = 0.94–1.05). Nevertheless, for the sake of looking for possible reasons that
might lead to such result, we performed heterogeneity analysis and tested publication bias. According to these results, 13 studies were excluded [29-31, 40, 43, 47, 48, 52, 55, 61, 63, 77, 107], the P-value was estimated to be 0.824, and the fixed effect model was applied. Ultimately, the results demonstrated that the rs1801133 polymorphism was significantly correlated with the risk of CRC (Figure 2) (dominant model: OR = 0.96, 95% CI = 0.94–0.99; recessive model: OR = 0.90, 95% CI = 0.83–0.96; homozygous model: OR = 0.88, 95% CI = 0.82–0.95; allele model: OR = 0.95, 95% CI = 0.93–0.98). All detailed results in the present meta-analysis are shown in Table 2.

In the subgroup analysis of ethnicity, MTHFR C677T polymorphism was found to reduce CRC susceptibility in Asians significantly (dominant model: OR = 0.94, 95% CI = 0.89–1.00 (Figure 3A); recessive model: OR = 0.88, 95% CI = 0.77–1.00; homozygous model: OR = 0.86, 95% CI = 0.75–1.00; allele model: OR = 0.92, 95% CI = 0.88–1.00). Simultaneously, significantly reduced risks were also found in mixed group (recessive model: OR = 0.83, 95% CI = 0.75–0.92; homozygous model: OR = 0.84, 95% CI = 0.75–0.93; allele model: OR = 0.95, 95% CI = 0.90–0.99). Amongst Caucasians, yet significantly reduced risks were only observed in the allele model (OR = 0.96, 95% CI = 0.93–1.00). Nevertheless, no significant associations were detected in Africans for all genetic models. When stratified by the source of controls, the positive results were observed in population-based control group (dominant model: OR = 0.97, 95% CI = 0.93–1.00 (Figure 3B); recessive model: OR = 0.88, 95% CI = 0.81–0.95; homozygous model: OR = 0.87, 95% CI = 0.80–0.93; allele model: OR = 0.95, 95% CI = 0.92–0.98). The similar significant associations were absent from hospital-based group for all the genetic models. The stratified analysis by genotyping methods showed that PCR-RFLP method (dominant model: OR = 0.95, 95% CI = 0.91–0.99 (Figure 3C); recessive model: OR = 0.90, 95% CI = 0.81–0.99; homozygous method: OR = 0.88, 95% CI = 0.79–0.97; allele model: OR = 0.95, 95% CI = 0.91–0.99) and Taqman method (recessive model: OR = 0.86, 95% CI = 0.73–1.00; homozygous method: OR = 0.85, 95% CI = 0.74–0.99; allele model: OR = 0.94, 95% CI = 0.89–0.99) were significantly correlated with risks of decreased CRC. However, RT-PCR method was not relevant to significant associations for all genetic models. In conclusion, the present meta-analysis suggested that MTHFR C677T polymorphism was connected with CRC susceptibility.

**Test of heterogeneity**
Heterogeneity analysis was performed in this meta-analysis, and heterogeneity was significantly observed between all the included studies in the dominant model ($I^2 = 62.0\%$, $P < 0.001$; Figure 4A). In addition, the Galbraith radial plot illustrated heterogeneity obviously. Meanwhile, it specifically pointed out 13 studies that might have led to the obvious heterogeneity and insignificant results of the meta-analysis [27, 29, 38, 41, 45, 46, 50, 53, 59, 61, 75, 105]. After excluding 13 studies, the heterogeneity decreased significantly ($I^2 = 0.0\%$, $P = 0.789$; Figure 4B) in the present meta-analysis.

**Publication bias**
The Begg's funnel plot and Egger's test were performed to assess the publication bias. Initially, the Begg's funnel plot was asymmetrical obviously with all the included studies and it suggested a potential publication bias (Begg's test: $P = 0.103$; Egger's test: $P = 0.058$; Figure 5A). After the removal of 13 studies mentioned above [27, 29, 38, 41, 45, 46, 50, 53, 59, 61, 75, 105], the plots seemed to have a symmetrical distribution in the funnel plot and then Egger's test was used to provide statistical evidence (Begg's test: $P = 0.369$; Egger's test: $P = 0.136$; Figure 5B). No significant publication bias was observed in the present studies.

**Sensitivity analysis**
In order to distinguish the impact of each study on the pooled ORs, we conducted one-way sensitivity analysis. Each time one study was omitted, meta-analysis was repeated and the statistical significance of the results was not changed. Therefore, the results confirmed that the present meta-analysis was relatively stable and reliable.

**Discussion**
MTHFR is a key enzyme in the folate metabolism and may play a role in the CRC carcinogenesis. It is an essential enzyme in the catalytic reaction that converts 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate. On one hand, 5,10-methylenetetrahydrofolate takes part in the thymidylate synthesis. On the other hand, 5-methyltetrahydrofolate promotes methionine synthesis and SAM-mediated methylations. In brief, MTHFR has an influence on DNA synthesis, methylation, and repair [113]. The MTHFR polymorphisms result in the decreased enzyme activity and then low levels of plasma folate and high homocysteine come to light. Folate is one of water-soluble B vitamins that takes part in various biochemical reactions with its activity to provide or accept one-carbon units [13]. Folate deficiency is likely to contribute to the development of CRC, and several mechanisms may explain how it leads to CRC, including DNA strand breaks, abnormal DNA methylation, and impaired DNA repair [114].
Figure 2. Forest plots of the association between MTHFR C677T polymorphism and CRC susceptibility in dominant model after omitting these 13 studies with heterogeneity and publication bias.
Table 2 Meta-analysis results for the included studies of the association between MTHFR rs1801133 polymorphism and risk of CRC

| Variables                        | Number of studies | OR (95% CI) Dominant model | P-values | I-squared (%) | OR (95% CI) Recessive model | P-values | I-squared (%) | OR (95% CI) Homozygous model | P-values | I-squared (%) | OR (95% CI) Heterozygous model | P-values | I-squared (%) | OR (95% CI) Allele model | P-values | I-squared (%) |
|----------------------------------|-------------------|-----------------------------|----------|---------------|-----------------------------|----------|---------------|-----------------------------|----------|---------------|-----------------------------|----------|---------------|-----------------------------|----------|---------------|
| rs1801133C>T                    |                   |                             |          |               |                             |          |               |                             |          |               |                             |          |               |                             |          |               |
| All                              | 78                | 0.96 (0.94–0.99)            | 0.824    | 0.0           | 0.90 (0.83–0.96)            | <0.001   | 49.9          | 0.88 (0.82–0.95)            | <0.001   | 42.5          | 0.99 (0.96–1.02)            | 0.0      | 0.95          | 0.006 (0.93–0.98)          | 31.2     |               |
| Ethnicity                        |                   |                             |          |               |                             |          |               |                             |          |               |                             |          |               |                             |          |               |
| Asian                            | 33                | 0.94 (0.89–1.00)            | 0.418    | 3.0           | 0.88 (0.77–1.00)            | 0.001    | 51.2          | 0.86 (0.75–1.00)            | 0.001    | 49.2          | 0.96 (0.91–1.02)            | 0.0      | 0.94          | 0.002 (0.88–1.00)          | 47.9     |               |
| Caucasian                        | 36                | 0.97 (0.93–1.01)            | 0.711    | 0.0           | 0.93 (0.83–1.04)            | <0.001   | 57.8          | 0.91 (0.82–1.01)            | 0.001    | 47.7          | 0.99 (0.95–1.03)            | 0.0      | 0.96          | 0.079 (0.93–1.00)          | 26.2     |               |
| African                          | 3                 | 0.98 (0.67–1.42)            | 0.866    | 0.0           | 0.69 (0.24–2.03)            | 0.873    | 0.0           | 0.72 (0.24–1.15)            | 0.837    | 0.0           | 1.02 (0.69–1.51)            | 0.0      | 0.93          | 0.816 (0.67–1.30)          | 0.0      |               |
| Mixed                            | 6                 | 0.98 (0.92–1.04)            | 0.959    | 0.0           | 0.83 (0.75–0.92)            | 0.829    | 0.0           | 0.84 (0.75–0.93)            | 0.830    | 0.0           | 1.02 (0.95–1.09)            | 0.0      | 0.95          | 0.908 (0.90–0.99)          | 0.0      |               |
| Source of control                |                   |                             |          |               |                             |          |               |                             |          |               |                             |          |               |                             |          |               |
| HB                               | 28                | 0.96 (0.90–1.03)            | 0.357    | 7.2           | 0.97 (0.81–1.16)            | <0.001   | 59.6          | 0.96 (0.80–1.05)            | <0.001   | 54.4          | 0.98 (0.92–1.04)            | 0.0      | 0.97          | 0.007 (0.90–1.05)          | 44.4     |               |
| PB                               | 50                | 0.97 (0.93–1.00)            | 0.911    | 0.0           | 0.88 (0.81–0.95)            | 0.001    | 43.3          | 0.87 (0.80–0.93)            | 0.012    | 34.1          | 0.99 (0.96–1.03)            | 0.0      | 0.95          | 0.087 (0.92–0.98)          | 22.4     |               |
| Geotyping                        |                   |                             |          |               |                             |          |               |                             |          |               |                             |          |               |                             |          |               |
| Taqman                           | 14                | 0.96 (0.92–1.01)            | 0.568    | 0.0           | 0.86 (0.73–1.00)            | <0.001   | 65.0          | 0.85 (0.74–1.00)            | 0.004    | 57.3          | 0.99 (0.94–1.05)            | 0.0      | 0.94          | 0.085 (0.89–0.99)          | 36.4     |               |
| PCR-RFLP                         | 50                | 0.95 (0.91–0.99)            | 0.886    | 0.0           | 0.90 (0.81–0.99)            | 0.001    | 43.6          | 0.98 (0.79–0.97)            | 0.005    | 37.5          | 0.93 (0.94–1.03)            | 0.0      | 0.95          | 0.027 (0.91–0.99)          | 30.0     |               |
| RT-PCR                           | 4                 | 1.10 (0.97–1.26)            | 0.746    | 0.0           | 1.12 (0.76–1.64)            | 0.017    | 70.4          | 1.15 (0.79–1.66)            | 0.042    | 63.4          | 1.11 (0.96–1.27)            | 0.0      | 1.08          | 0.207 (0.95–1.22)          | 34.2     |               |

These 13 studies by Ozen et al., Ashmore et al., Delgado-Plasencia et al., Zhu et al., Prasad et al., Komlosi et al., Karpinski et al., Naghibalhossaini et al., Fernández-Peralta et al., Awady et al., Haghighi et al., Jin et al., Ryan et al. were removed [29, 30, 31, 40, 43, 47, 48, 52, 55, 61, 63, 77, 107].
Several polymorphisms have been reported about the MTHFR gene coding relevant enzyme, and MTHFR C677T polymorphism is the most common one. Heretofore, various studies conducted to detect such association and obtained inconsistent results. Chen et al. [112], first reported that MTHFR variant homozygous (TT) genotype was closely linked to reduced incidence of CRC with low consumption of alcohol. In the next few years, similar results were replicated by several other studies [109-111]. However, another study of a homogeneous northern European population obtained different conclusions that MTHFR CT heterozygote had a significantly increased risk of developing CRC and no increased cancer risk was observed in TT homozygotes [107]. In addition, a hospital-based case–control study conducted by Matsuo et al. [104] found no significant relativity between MTHFR C677T and the
risks of CRC. Owing to the difference in study design and the sample size, the different ethnicity, and the diverse stratification, these controversial results were found in published studies. Hence, meta-analysis is essential to be carried out by combining all studies that meet the requirements to get more precise conclusions.

In recent years, there were several meta-analyses performed to elucidate the association of MTHFR C677T polymorphism and the susceptibility to CRC before [26,115-118]. Compared with them, this meta-analysis included the most eligible reported studies with the largest sample size and had no restrictions in ethnicity. Since the quality of included documents were disequilibrium, our initial analysis achieved no significant results with all eligible studies. In order to obtain more reliable results, the final conclusion were obtained excluding 13 studies in accordance with the analysis of heterogeneity and publication bias. In this meta-analysis, the pooled conclusions revealed that rs1801133 polymorphism significantly reduced the risk of CRC in the dominant model. The findings agreed with the overwhelming majority results reported by the published studies.

When stratified by ethnicity, there was a weak association with reduced risks of CRC in Asians. The result was consistent with the two previous meta-analysis based on the Asians [116,117]. Zhong et al. [118], carried out a meta-analysis obtaining similar results in East Asians and further subgroup analyses by country identified such association in Korea and Japan. Nevertheless, the recent meta-analysis failed to identify that rs1801133 polymorphism was connected with CRC susceptibility in Iranian population [26]. By means of stratified analysis based on the source of controls and genotyping methods, the positive results were observed in population-based control group and PCR-RFLP method. In general, the source of controls included healthy individuals and patients without CRC. Since the risks of CRC varies amongst individuals over a few years, it might have an impact on the results of relevant studies and make them unreliable. Therefore, inclusion criteria should be improved and studies with large sample sizes should be accepted. In the subgroup of genotyping method, there were nine methods applied for genotyping such as PCR-RFLP, RT-PCR, PCR-SSCP, MS-PCR, MSP, MALDI-TOF-MS, Taqman, MassARRAY, and Sequenom in the including studies. Specific methods and steps were described in each article. Amongst these 87 studies, the majority method was PCR-RFLP. Different methods have their own merits, and when all included studies used the same method, the final results would be more reliable.

In the present meta-analysis, we had obtained weak associations significantly with a large sample size. However, the potential limitations of the meta-analysis should be acknowledged. First, this meta-analysis was based on unadjusted effect estimates and 95% CI, and the influence of multiple cofactors such as age, gender, diet habits including intake of alcohol and consumption of cigarette, the level of folate, and the other environmental factors should be taken into consideration. Second, because of incomplete data of some genotypes, only the dominant model was analyzed in all the included studies. Third, we did not perform stratification analysis by serum folate levels, locations of the tumor and so on, which might result in confounding bias. In addition, after excluding 13 studies according to the analysis of heterogeneity and publication bias, the heterogeneity decreased significantly and the publication bias seemed to disappear. However, the selection bias existed because all the studies were published. Furthermore, the gene–gene and gene–environment interactions were not mentioned in this meta-analysis. In addition, the potential roles of the gene polymorphism which were hidden or magnified by other interactions were omitted.
Conclusion
In summary, the present meta-analysis revealed that there was a significant association between MTHFR C677T polymorphism and susceptibility to CRC. Simultaneously, the TT genotype of MTHFR C677T polymorphism could reduce the risk of CRC. In addition, the associated risk of CRC was also reduced in Asians and those studies with population-based controls and used the PCR-RFLP method. Therefore, detection of the MTHFR C677T polymorphism might be used as markers for CRC prediction and treatment selection.

Competing interests
The authors declare that there are no competing interests associated with the manuscript.

Funding
This work has been supported by the Natural Science Funding of Jiangsu Province [grant number BK20141492]; and the ‘333 Project’ of Jiangsu Province [grant number BRA2016517].

Author contribution
Y.G., H.Y., and Z.Q. were responsible for conception and design. Y.G., H.Y., and F.W. provided the conceptual support. S.S., Z.G., and L.L. were responsible for the collection and assembly of data. P.L., X.H., and X.C. were responsible for data analysis and interpretation. L.X., Z.Q., and F.W. were responsible for manuscript writing. All the authors approved the final manuscript.

Abbreviations
CI, confidence interval; CRC, colorectal cancer; HWE, Hardy–Weinberg equilibrium; MSP, mutagenically separated PCR; MS-PCR, methylation-specific PCR; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; PCR-RFLP, PCR-restriction fragment length polymorphism; PCR-SSCP, PCR-single strand conformation polymorphism; PRISMA-P, preferred reporting items for systematic review and meta-analysis protocol; RT-PCR, real-time PCR; SAM, S-adenosylmethionine.

References
1 Siegel, R.L., Miller, K.D. and Jemal, A. (2016) Cancer statistics, 2016. CA Cancer J. Clin. 66, 7–30
2 Markowitz, S.D. and Bertagnolli, M.M. (2009) Molecular basis of colorectal cancer. N. Engl. J. Med. 361, 2449–2460
3 Baroudi, O. and Benammar-elgaaied, A. (2016) Involvement of genetic factors and lifestyle on the occurrence of colorectal and gastric cancer. Crit. Rev. Oncol. Hemat. 107, 72–81
4 Khan, A.A., Khan, Z., Malik, A., Kalam, A.M., Cash, P., Ashraf, T.M. et al. (2017) Colorectal cancer-inflammatory bowel disease nexus and felony of Escherichia coli. Life Sci. 180, 60–67
5 Nasiiri, M., Kooshyar, M.M., Roudbar, Z., Mahdavi, M. and Doosti, M. (2013) Genes and SNPs associated with non-hereditary and hereditary colorectal cancer. Asian Pac. J. Cancer Prev. 14, 5609–5614
6 Noci, S., Dugo, M., Bertola, F., Melotti, F. and Vannelli, A. (2016) A subset of genetic susceptibility variants for colorectal cancer also has prognostic value. Pharmacogenomics J. 16, 173–179
7 Guo, X.P., Wang, Y., Zhao, H., Song, S.D., Zhou, J. and Han, Y. (2014) Association of MTHFR C677T polymorphisms and colorectal cancer risk in Asians: evidence of 12,255 subjects. Clin. Transl. Oncol. 16, 623–629
8 Fang, X., Xu, W., Huang, Q., Yang, X.K., Liu, Y.Y., Leng, R.X. et al. (2014) 5,10-Methylenetetrahydrofolate reductase polymorphisms and colon cancer risk: a meta-analysis. Asian Pac. J. Cancer Prev. 15, 8245–8250
9 Ueland, P.M., Hustad, S., Schneede, J., Refsum, H. and Vollset, S.E. (2001) Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol. Sci. 22, 195–201
10 Goyette, P., Pai, A., Milos, R., Frostell, P., Tran, P., Chen, Z.T. et al. (1998) Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm. Genome 9, 652–659
11 Rozen, R. (1997) Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). Thromb. Haemost. 78, 523–526
12 Frostell, P., Milos, R., Goyette, P., Sheppard, C.A., Matthews, R.G., Boers, G.J.H. et al. (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature 10, 111–113
13 Duthie, S.J. (1999) Folic acid deficiency and cancer: mechanisms of DNA instability. Br. Med. Bull. 55, 578–592
14 Zhu, X.L., Liu, Z.Z., Yan, S.X., Wang, W., Chang, R.X., Zhang, Y.C. et al. (2016) Association between the MTHFR A1298C polymorphism and risk of cancer: evidence from 265 case-control studies. Mol. Genet. Genomics 291, 51–63
15 Long, S. and Goldblatt, J. (2016) MTHFR genetic testing: controversy and clinical implications. Aust. Fam. Physician 45, 237–240
16 Liew, S. and Gupta, E.D. (2015) Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: Epidemiology, metabolism and the associated diseases. Eur. J. Med. Genet. 58, 1–10
17 Shi, H., Yang, S.W., Liu, Y., Huang, P., Lin, N., Sun, X.R. et al. (2015) Study on environmental causes and SNPs of MTHFR, MS and CBS genes related to congenital heart disease. PLoS ONE 10, e128646
18 Sohda, S., Arinami, T., Hamada, H., Yamada, N. and Hamaguchi, H. (1997) Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia. J. Med. Genet. 34, 525–526
19 Stonek, F. et al. (2007) Methylenetetrahydrofolate reductase C677T polymorphism and pregnancy complications. Obstet. Gynecol. 110, 363–368
20 van der Put, N.M.J., Gabreels, F., Stevens, E.M.B., Smeitink, J.A.M., Trijpebs, F.J., Eskes, T.K.A.B. et al. (1998) A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am. J. Hum. Genet. 62, 1044–1051
21 Shiao, S.P.K. and Yu, C.H. (2016) Meta-prediction of MTHFR gene polymorphism mutations and associated risk for colorectal cancer. Biol. Res. Nura. 18, 357–369
22 Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M. et al. (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P): elaboration and explanation. BMJ 349, g6747
23 Wei, G.S. and Thompson, E.A. (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48, 361–372
24 Anzuera-Cabrera, J. and Higgins, J.P. (2010) Graphical displays for meta-analysis: an overview with suggestions for practice. Res. Synth. Methods 1, 66–80
25 Hayashino, Y., Noguchi, Y. and Fukui, T. (2005) Systematic evaluation and comparison of statistical tests for publication bias. J. Epidemiol. 15, 235–243
26 Haerian, M.S., Haerian, B.S., Molanaei, S., Kosari, F., Sabeti, S., Bidari-Zerepoosh, F. et al. (2016) MTHFR rs1801133 polymorphism and susceptibility to colorectal cancer in Iranian population: evidence of a case-control study and meta-analysis. Pharmacogenomogenics 17, 1957–1965
27 Kim, J.W., Joon, J.Y., Jang, M.J., Kim, J.O. and Chong, S.Y. (2015) Association between folate metabolism-related polymorphisms and colorectal cancer risk. Mol. Clin. Oncol. 3, 639–648
28 Rai, P.S., Pai, G.C., Alvaress, J.F., Bellampalli, R., Gopinath, P.M. and Satyamoorthy, k. (2014) Intraindividual somatic variations in MTHFR gene polymorphisms in relation to colon cancer. Pharmacogenomics 15, 349–359
29 Ozen, F., Sen, M. and Ozdemir, O. (2014) Methylenetetrahydrofolate reductase gene germ-line C677T and A1298C SNPs are associated with colorectal cancer risk in the Turkish population. Asian Pac. J. Cancer Prev. 15, 7731–7735
30 Ashmore, J.H., Lesko, S.M., Muscat, J.E., Gallilher, C.J., Berg, A.S., Miller, P.E. et al. (2013) Association of dietary and supplemental folate intake and polymorphisms in three FOCM pathway genes with colorectal cancer in a population-based case-control study. Gene. Chromosome Canc. 52, 945–953
31 Delgado-Plascencia, L., Medina-Aranza, V., Bravo-Gutierrez, A., Perez-Palma, J., Alvarez-Aguuelas, H., Salido-Ruiz, E. et al. (2013) Impact of the MTHFR C677T polymorphism on colorectal cancer in a population with low genetic variability. Int. J. Colorectal Dis. 28, 1187–1193
32 Yousef, A., Shamaf, M., Berger, S., Ababneh, N., Bobai, Y., Al, D. et al. (2013) Allele and genotype frequencies of the polymorphic methylenetetrahydrofolate reductase and colorectal cancer among Jordanian population. Asian Pac. J. Cancer Prev. 14, 4559–4565
33 Lee, J.E., Wei, E.K., Fuchs, C.S., Hunter, D.J., Lee, I.M., Selhub, J. et al. (2012) Plasma folate, methylenetetrahydrofolate reductase (MTHFR), and colorectal cancer risk in three large nested case-control studies. Cancer Cause Control 23, 537–545
34 Promthet, S., Pientong, C., Ekalaksananan, T., Songsenn, N., Poomphakwaen, k., Chopijitt, P. et al. (2012) Risk factors for rectal cancer and methylenetetrahydrofolate reductase polymorphisms in a population in northeast Thailand. Asian Pac. J. Cancer Prev. 13, 4017–4023
35 Kim, J., Cho, Y.A., Kim, D.H., Lee, B.H., Hwang, D.Y., Jeong, J. et al. (2012) Dietary intake of folate and alcohol, MTHFR C677T polymorphism, and colorectal cancer risk in Korea. Am. J. Clin. Nutr. 95, 405–412
36 Yin, G., Ming, H., Zheng, X., Xuan, Y., Liang, J. and Jin, X. (2012) Methylenetetrahydrofolate reductase C677T gene polymorphism and colorectal cancer risk: a case-control study. Oncol. Lett. 4, 365–369
37 Sameer, A.S., Shah, Z.A., Nissar, S., Mudassar, S. and Siddiqi, M.A. (2011) Risk of colorectal cancer associated with the methylenetetrahydrofolate reductase C677T polymorphism in the Kashmiri population. Genet. Mol. Res. 10, 1200–1210
38 Vossen, C.Y., Hofmeister, M., Chang-Cladue, J.C., Rosendaal, F.R. and Brenner, H. (2011) Clotting factor gene polymorphisms and colorectal cancer risk. J. Clin. Oncol. 29, 1722–1727
39 Kang, B.S., Ahn, D.H., Kim, N.K. and Kim, J.W. (2011) Relationship between metabolic syndrome and MTHFR polymorphism in colorectal cancer. J. Korean Soc. Coloproctol. 27, 78–82
40 Zhu, Q., Jin, Z., Yuan, Y., Lu, Q., Ge, D. and Zong, M. (2011) Impact of MTHFR gene C677T polymorphism on Bcl-2 gene methylation and protein expression in colorectal cancer. Scand. J. Gastroenterol. 46, 436–445
41 Pardini, B., Kumar, R., Naccarati, A., Prasad, R.B., Forstl, A., Polakova, V. et al. (2011) MTHFR and MTRR genotype and haplotype analysis and colorectal cancer susceptibility in a case-control study from the Czech Republic. Mutat. Res. 721, 74–80
42 Kim, J.W., Park, H.M., Choi, Y.K., Chong, S.Y. and Oh, D. (2011) Polymorphisms in genes involved in folate metabolism and plasma DNA methylation in colorectal cancer patients. Oncol. Rep. 25, 167–172
43 Prasad, V.V.T.S. and Wilkook, H. (2011) Association of the functional polymorphism C677T in the methylenetetrahydrofolate reductase gene with colorectal, thyroid, breast, ovarian, and cervical cancers. Onkologie 34, 422–426
44 Li, H., Xu, X.W., Shen, H.L., Chen, Q.Y., Hui, L.L., Long, L.L. et al. (2011) Methylenetetrahydrofolate reductase genotypes and haplotypes associated with susceptibility to colorectal cancer in an eastern Chinese Han population. Genet. Mol. Res. 10, 3738
45 Jokic, M., Bricic-Kosti, K., Stefullj, J., Ivkovic, T.C., Bozo, L., Gamulin, M. et al. (2011) Association of MTHFR, MTR, MTRR, RFC1, and DHFR gene polymorphisms with susceptibility to sporadic colon cancer. DNA Cell Biol. 30, 771–776
46 Giumaar, J.L.M., Ayrizono, M.D.L., Coy, C.S.R. and Lima, C.S.P. (2011) Gene polymorphisms involved in folate and methionine metabolism and increased risk of sporadic colorectal adenocarcinoma. Tumor Biol. 32, 853–861
47 Komlosi, V., Hitre, E., Pap, E., Adeff, V., Reti, A., Szekely, E. et al. (2010) SHMT1 1420 and MTHFR 677 variants are associated with rectal but not colon cancer. BMC Cancer 10, 1471–2407
48 Karpinski, P., Myszka, A., Ramsey, D., Misiak, B., Gil, J., Laczmanska, I. et al. (2010) Polymorphisms in methyl-group metabolism genes and risk of sporadic colorectal cancer with relation to the CpG island methylator phenotype. *Cancer Epidemiol.* 34, 338–344

49 Cui, L., Shiu, M., Kweon, S., Kim, H.N., Song, H., Piao, J. et al. (2010) Methylene tetrahydrofolate reductase C677T polymorphism in patients with gastric and colorectal cancer in a Korean population. *BMC Cancer* 10, 1471–1477

50 Eussen, S.J.P.M., Vollset, S.E., Iglind, J., Meyer, K., Fredriksen, A., Ueland, P.M. et al. (2010) Plasma folate, related genetic variants, and colorectal cancer risk in EPIC. *Cancer Epidemiol. Biomarkers Prev.* 19, 1328–1340

51 Chandy, S., Adiga, M.N.S., Ramachandra, N., Krishnamoorthy, S., Ramaswamy, G., Savithri, H.S. et al. (2010) Association of methylenetetrahydrofolate reductase gene polymorphisms & colorectal cancer in India. *Indian J. Med. Res.* 131, 659–664

52 Naghibalhossaini, F., Mokarram, P., Khalli, I., Vaseli, M., Hosseini, S.V., Ashktorab, H. et al. (2010) *MTHFR* C677T and A1298C variant genotypes and the risk of microsatellite instability among Iranian colorectal cancer patients. *Cancer Genet. Cyto. Genet.* 197, 142–151

53 Promthet, S.S., Pientong, C., Ekalaksananan, T., Wangnon, S., Poompakhwan, K., Songserm, N. et al. (2010) Risk factors for colon cancer in northeastern Thailand: interaction of *MTHFR* codon 677 and 1298 genotypes with environmental factors. *J. Epidemiol.* 20, 329–338

54 Yang, X.X., Li, F.X., Yi, J.P., Li, X., Sun, J.Z. and Hu, N.Y. (2010) Impact of methylenetetrahydrofolate reductase C677T polymorphism on the risk of gastric cancer, colorectal cancer and lung cancer. *Gangdong Med.* 31, 2375–2378

55 Fernández-Peralta, A.M., Daimiel, L., Nejda, N., Iglesias, D., Medina Arana, V. and González-Aguilera, J.J. (2010) Association of polymorphisms *MTHFR* C677T and A1298C with risk of colorectal cancer, genetic and epigenetic characteristic of tumors, and response to chemotherapy. *Int. J. Colorectal Dis.* 25, 141–151

56 Zhu, F., Wang, Y.-m. and ZhangQY, Q.-y. (2010) A case-control study of plasma homocysteine, serum folate, the polymorphism of methylenetetrahydrofolate reductase in colorectal cancer. *J. Southeast Univ. Med. Sci. Edi.* 25, 1328–1340

57 Iacopetta, B., Heyworth, J., Girschik, J., Grieu, C., Clayforth, C. and Fritschi, L. (2009) The *MTHFR* C677T and ΔDNMT3B C-149T polymorphisms confer different risks for right- and left-sided colorectal cancer. *Int. J. Cancer* 125, 84–90

58 Gallegos-Arreola, M.P., García-Ortíz, J.E., Figuera, L.E., Puebla-Perez, A.M., Morgan-Villela, G., Zuniga-Gonzalez, G.M. et al. (2009) Association of the 677C→T polymorphism in the *MTHFR* Gene with Colorectal cancer in Mexican patients. *Cancer Genome Proteomics* 6, 183–188

59 Reeves, S.G., Meldrum, C., Groomebridge, C., Spigelman, A.D., Suchy, J., Kurzawski, G. et al. (2009) MTHFR 677 C→T and 1298 A→C polymorphisms and the age of onset of colorectal cancer in hereditary nonpolyposis colorectal cancer. *Eur. J. Hum. Genet.* 17, 629–635

60 El Awady, M.K., Karim, A.M., Hanna, L.S., El Husseiny, L.A., El Sahar, M., Abdel Menem, H.A. et al. (2009) Methylenetetrahydrofolate reductase gene polymorphisms and the risk of colorectal carcinoma in a sample of Egyptian individuals. *Cancer Biomark.* 5, 233–240

61 Derwinger, K., Wettergren, Y., Odin, E., Carlsson, G. and Gustavsson, B. (2009) A study of the *MTHFR* gene polymorphism C677T in colorectal cancer. *Clin. Colorectal Cancer* 8, 43–48

62 Theodoratou, E., Farrington, S.M., Tenesa, A., McNeill, G., Cetnarskyj, R., Barnetson, R.A. et al. (2008) Dietary vitamin B6 intake and the risk of colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.* 17, 183–188

63 Sharp, L., Little, J., Brockton, N.T., Cotton, S.C., Masson, L.F., Haites, N.E. et al. (2008) Polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR*) gene, intakes of folate and related B vitamins and colorectal cancer: a case-control study in a population with relatively low folate intake. *Br. J. Nutr.* 99, 379–389

64 Kury, S., Buecher, B., Robiou-du-Pont, S., Scoul, C., Colman, H., Neel, T.L. et al. (2008) Low-penetrance alleles predisposing to sporadic colorectal cancers: a French case-controlled genetic association study. *BMC Cancer* 8, 326

65 El Awady, M.K., Karim, A.M., Hanna, L.S., El Husseiny, L.A., El Sahar, M., Abdel Menem, H.A. et al. (2009) Methylenetetrahydrofolate reductase gene polymorphisms and the risk of colorectal carcinoma in sporadic colorectal cancer through an interaction with folate/vitamin B12 status. *World J. Gastroenterol.* 14, 3662

66 Cao, H., Gao, C., Takezaki, T., Wu, J., Ding, J., Liu, Y. et al. (2008) Genetic polymorphisms of methylenetetrahydrofolate reductase and susceptibility to colorectal cancer. *Asian Pac. J. Cancer Prev.* 9, 203–208

67 Theodoratou, E., Farrington, S.M., Tenesa, A., McNeill, G., Cetnarskyj, R., Barnetson, R.A. et al. (2008) Dietary vitamin B6 intake and the risk of colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.* 17, 171–182

68 Eklof, V., Van Guelpen, B., Hultdin, J., Johansson, I., Hallmans, G. and Palmqvist, R. (2009) The reduced folate carrier (*FRCT1*) 80G→A and folate hydrolase 1 (*FOLH1*) 1561C→T polymorphisms and the risk of colorectal cancer: a nested case-referent study. *Scand. J. Clin. Lab. Inv.* 68, 393–401

69 Zhang, Y.L., Yuan, X.Y., Zhang, C., Yang, Y., Pan, Y.M., Zhou, Z.Y. et al. (2006) Relationship between polymorphisms of thymidylate synthase and methylenetetrahydrofolate reductase and susceptibility in Lianoning Benxi colorectal cancer patients. *Cancer J. Clin.* 13, 769–773

70 Guerreiro, C.S., Carmona, B., Gonçalves, S., Carolino, E., Fidalgo, P., Brito, M. et al. (2008) Risk of colorectal cancer associated with the C677T polymorphism in 5,10-methylenetetrahydrofolate reductase in Portuguese patients depends on the intake of methyl-donor nutrients. *Am. J. Clin. Nutr.* 88, 1413–1418

71 Osian, G., Procopciuc, L. and Vlad, L. (2007) *MTHFR* polymorphisms as prognostic factors in sporadic colorectal cancer. *J. Gastrointestin. Liver Dis.* 16, 251–256

72 Zeybek, U., Yaylim, I., Yilmaz, H., Agachan, B., Ergen, A., Arikan, S. et al. (2007) Methylenetetrahydrofolate reductase C677T polymorphism in patients with gastric and colorectal cancer. *Cell Biochem. Funct.* 25, 419–422

73 Lima, C.S.P., Nascimento, H., Bonadia, L.C., Teori, M.T., Coy, C.S.R., Goes, J.R.N. et al. (2007) Polymorphisms in methylenetetrahydrofolate reductase gene (*MTHFR*) and the age of onset of sporadic colorectal adenocarcinoma. *Int. J. Colorectal Dis.* 22, 757–763
14 Chang, S., Lin, P., Lin, J., Yang, S., Wang, H. and Li, A. (2007) Role of MTHFR polymorphisms and folate levels in different phenotypes of sporadic colorectal cancers. Int. J. Colorectal Dis. 22, 483–489
15 Murtaugh, M.A., Curtin, K., Sweeney, C., Wolff, R.K., Holubkov, R. and Caan, B.J. (2007) Dietary intake of folate and co-factors in folate metabolism, MTHFR polymorphisms, and reduced rectal cancer. Cancer Cause Control 18, 153–163
16 Jin, X.X., Zhu, Z.Z., Wang, A.Z. and Jia, H.R. (2007) Association of methylenetetrahydrofolate reductase C677T polymorphism with genetic susceptibility to colorectal cancer. World J. Dig. 15, 2754–2757
17 Curtin, K., Slattery, M.L., Ulrich, C.M., Bigler, J., Levin, T.R., Wolff, R.K. et al. (2007) Genetic polymorphisms in one-carbon metabolism: associations with CpG island methylator phenotype (CIMP) in colon cancer and the modifying effects of diet. Carcinogenesis 28, 1672–1679
18 Hubner, R.A., Lubble, S., Chandler, I. and Houlston, R.S. (2007) MTHFR C677T has differential influence on risk of MSI and MSS colorectal cancer. Hum. Mol. Genet. 16, 1072–1077
19 Koushik, A., Kraft, P., Fuchs, C.S., Hankinson, S.E., Willett, W.C., Giovannucci, E.L. et al. (2006) Nonsynonymous polymorphisms in genes in the one-carbon metabolism pathway and associations with colorectal cancer. Cancer Epidemiol. Biomarkers Prev. 15, 2408–2417
20 Battistelli, S., Vittoria, A., Stefanoni, M., Bing, C. and Roviello, F. (2006) Total plasma homocysteine and methylenetetrahydrofolate reductase C677T polymorphism in patients with colorectal carcinoma. World J. Gastroenterol. 12, 6128–6132
21 Van Guelpen, B., Hultdin, J., Johansson, I., Hallmans, G., Stenling, R., Riboli, E. et al. (2006) Low folate levels may protect against colorectal cancer. Gut 55, 1461–1466
22 Wang, J., Gajalakshmi, V., Jiang, J., Kuriki, K., Suzuki, S., Nagaya, T. et al. (2006) Associations between 5,10-methylenetetrahydrofolate reductase codon 677 and 1298 genetic polymorphisms and environmental factors with reference to susceptibility to colorectal cancer: a case-control study in an Indian population. Int. J. Cancer 118, 991–997
23 Chen, K., Song, L., Jin, M.J., Fang, C.H., Jiang, X.D. and Yu, W.P. (2006) Associations between folate metabolism enzyme gene polymorphisms and colorectal susceptibility. Chin. J. Oncol. 28, 429–432
24 Matsuo, K., Ito, H., Wakai, K., Hirose, K., Saito, T., Suzuki, T. et al. (2005) One-carbon metabolism related gene polymorphisms interact with alcohol drinking to influence the risk of colorectal cancer in Japan. Carcinogenesis 26, 2164–2171
25 Landi, S., Gemignani, F., Moreno, V., Gioia-Patrincola, L., Chabrier, A., Guino, E. et al. (2005) A comprehensive analysis of phase I and phase II metabolism gene polymorphisms and risk of colorectal cancer. Pharmacogenet. Genomics 15, 535–546
26 Le Marchand, L., Wilkens, L.R., Kolonel, L.N. and Henderson, B.E. (2005) The MTHFR C677T polymorphism and colorectal cancer: the multiethnic cohort study. Cancer Epidemiol. Biomarkers Prev. 14, 1198–1203
27 Jiang, Q., Chen, K., Ma, X.Y., Yao, K.Y., Yu, W.P., Li, Y.Y. et al. (2005) Diets, polymorphisms of methylenetetrahydrofolate reductase, and the susceptibility of colon cancer and cancer. Cancer Detect. Prev. 29, 146–154
28 Otani, T., Iwasaki, M., Hanoaka, T., Kobayashi, M., Ishihara, J., Natsukawa, S. et al. (2005) Folate, vitamin B6, vitamin B12, and vitamin B2 intake, genetic polymorphisms of related enzymes, and risk of colorectal cancer in a hospital-based case-control study in Japan. Nutr. Cancer 53, 42–50
29 Miso, X.P., Yang, S., Tan, W., Zhang, X.M., Ye, Y.J., Lin, Y.J. et al. (2005) Association between genetic variations in methylenetetrahydrofolate reductase and risk of colorectal cancer in a Chinese population. Chin. Prev. Med. 39, 409–411
30 Kim, D., Ahn, Y., Lee, B., Tsuji, E., Kiyohara, C. and Kono, S. (2004) Methylenetetrahydrofolate reductase polymorphism, alcohol intake, and risks of colon and rectal cancers in Korea. Cancer Lett. 216, 199–205
31 Ulvik, A., Vollset, S.E., Hansen, S., Gislefoss, R., Jellum, E. and Ueland, P.M. (2004) Colorectal cancer and the methylenetetrahydrofolate reductase 677C→T and methionine synthase 2756A→G polymorphisms: a study of 2,168 case-control pairs from the JANUS cohort. Cancer Epidemiol. Biomarkers Prev. 13, 2175–2180
32 Yin, G., Kono, S., Toyomura, K., Hagiwara, T., Nagano, J., Mizoue, T. et al. (2004) Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and colorectal cancer: the Fukuoka Colorectal Cancer Study. Cancer Sci. 95, 908–913
33 Curtin, K., Bigler, J., Slattery, M.L., Caan, B., Potter, J.D. and Ulrich, C.M. (2003) MTHFR C677T and A1298C polymorphisms: diet, estrogen, and risk of colon and rectal cancer. Cancer Epidemiol. Biomarkers Prev. 12, 285–292
34 Pufulete, M., Al-Ghnaniem, R., Leather, A.J.M., Appleby, P., Gout, S., Terry, C. et al. (2003) Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study. Gastroenterology 124, 1240–1248
35 Plasschke, J., Schwanebeck, U., Pistorius, S., Saeger, H.D. and Schackert, H.K. (2003) Methylenetetrahydrofolate reductase polymorphisms and risk of sporadic and hereditary colorectal cancer with or without microsatellite instability. Cancer Lett. 191, 179–185
36 Toffoli, G., Gafa, R., Russo, A., Lanza, G., Dolcetti, R., Sarfor, F. et al. (2003) Methylenetetrahydrofolate reductase 677 C→T polymorphism and risk of proximal colorectal cancer in north Italy. Clin. Cancer Res. 9, 743–748
37 Heljman, B.T., Boer, J.M.A., Suchiman, H.E.D., Cornilisse, C.J., Westendorp, R.G.J., Kromhout, D. et al. (2003) A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer. Cancer Res. 63, 1249–1253
38 Huang, P., Zhou, Z.Y., Ma, H.T., Liu, J.Y., Zhou, Y.H., Cao, J. et al. (2003) MTHFR polymorphisms and colorectal cancer susceptibility in Chongqing people. Acta Acad. Med. Mil. 26, 1704–1710
39 Barna, B., Erika, H., Vilmos, A., Ferenc, C., Fruzsina, G., Istvan, L. et al. (2004) A metilentetrahidrofolát-redukációz (MTHFR) C677T polymorfizmus klinikai jelentősége a metastatikus colorectalis daganatok 5-fluoropirimidin-álapú kezelésében. Magyar Onkológia 48, 253–257
40 Keker, T., Millikan, R., Worley, K., Keku, T., Millikan, R., Wilkens, L.R. and Seifried, A. (2002) B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). Cancer Cause Control 13, 239–248
41 Shannon, B., Gnanasampanthan, S., Beilby, J. and lacopetta, B. (2002) A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability. Gut 50, 520–524
Matsuo, K., Hamajima, N., Hirai, T., Kato, T. and Inoue, M. (2002) Methionine synthase reductase gene A66G polymorphism is associated with risk of colorectal cancer. *Asian Pac. J. Cancer Prev.* 3, 353–359

Sachse, C., Smith, G., Wilkie, M., Barrett, J.H. and Waxman, R. (2002) A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. *Carcinogenesis* 23, 1839–1849

Chen, J., Ma, J., Stampfer, M.J., Palomeque, C., Selhub, J. and Hunter, D.J. (2002) Linkage disequilibrium between the 677C>T and 1298A>C polymorphisms in human methylenetetrahydrofolate reductase gene and their contributions to risk of colorectal cancer. *Pharmacogenetics* 12, 339–342

Ryan, B.M., Molloy, A.M., McManus, R., Arfin, Q., Kelleher, D., Scott, J.M. et al. (2001) The methylenetetrahydrofolate reductase (MTHFR) gene in colorectal cancer. *Int. J. Gastrointestin. Cancer* 30, 105–111

Sloattery, M.L., Edwards, S.L., Samowitz, W. and Potter, J. (2000) Associations between family history of cancer and genes coding for metabolizing enzymes (United States). *Cancer Cause Control* 11, 799–803

Ryan, B.M., Molloy, A.M., McManus, R., Arfin, Q., Kelleher, D., Scott, J.M. et al. (2001) The methylenetetrahydrofolate reductase (MTHFR) gene in colorectal cancer. *Int. J. Gastrointestin. Cancer* 30, 105–111

Chen, J., Giovannucci, E. and Kelsey, K. (1996) A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res.* 56, 4862–4864

Zhou, D., Mei, Q., Luo, H., Tang, B. and Yu, P. (2012) The polymorphisms in methylenetetrahydrofolate reductase, methionine synthase, methionine synthase reductase, and the risk of colorectal cancer. *Int. J. Biol. Sci.* 8, 819–830

Kennedy, D.A., Stern, S.J., Matok, I., Moretti, M.E., Sarker, M., Adams-Webber, T. et al. (2012) Folate intake, MTHFR polymorphisms, and the risk of colorectal cancer: a systematic review and meta-analysis. *J. Cancer Epidemiol.* 2012, 952508

Haerian, B.S. and Haerian, M.S. (2015) Evaluation of association studies and meta-analyses of MTHFR gene polymorphisms in colorectal cancer. *Pharmacogenomics* 16, 413–425

Yang, Z., Zhang, X., Liu, H., Hao, Y. and Zhao, C. (2012) MTHFR C677T polymorphism and colorectal cancer risk in asians, a meta-analysis of 21 Studies. *Asian Pac. J. Cancer Prev.* 13, 1203–1208

Guo, X.P., Wang, Y., Zhao, H., Song, S.D., Zhou, J. and Han, Y. (2014) Association of MTHFR C677T polymorphisms and colorectal cancer risk in Asians: evidence of 12,255 subjects. *Clin. Transl. Oncol.* 16, 623–629

Zhong, S., Yang, J., Liu, K., Jiao, B.H. and Chang, Z. (2012) Quantitative assessment of the association between MTHFR C677T polymorphism and colorectal cancer risk in East Asians. *Tumor Biol.* 33, 2041–2051