Association of MTRR A66G polymorphism with cancer susceptibility: Evidence from 85 studies

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Abstract

Methionine synthase reductase (MTRR) is a key regulatory enzyme involved in the folate metabolic pathway. Previous studies investigating the association of MTRR A66G polymorphism with cancer susceptibility reported inconclusive results. We performed the current meta-analysis to obtain a more precise estimation of the possible association. Published literatures were identified from PubMed, Embase and CBM databases up to October 2016. The strength of the association between the MTRR A66G polymorphism and cancer susceptibility was assessed using odds ratios (ORs) and the corresponding 95% confidence intervals (CIs). Eighty five published studies with 32,272 cases and 37,427 controls were included in this meta-analysis. Pooled results indicated that the MTRR A66G polymorphism was associated with an increased overall cancer risk (homozygous model: OR = 1.08, 95% CI = 1.02-1.15, \( P = 0.009 \); recessive model: OR = 1.06, 95% CI = 1.00-1.12, \( P < 0.001 \) and allele comparison: OR = 1.03, 95% CI = 1.00-1.06, \( P < 0.001 \)). Stratification analysis further indicated significant associations in head and neck cancer, Caucasians, Africans, and high quality studies. However, to avoid the “false-positive report”, the significant findings were assessed by the false-positive report probability (FPRP) test. Interestingly, the results of FPRP test revealed that the increased risk for MTRR A66G polymorphism among Africans need further validation due to the high probabilities of false-positive results. This meta-analysis suggests that the MTRR A66G polymorphism is associated with significantly increased cancer risk, a finding that needs to be confirmed in single large studies.

Key words: Methionine synthase reductase (MTRR); polymorphism; susceptibility; meta-analysis.

Introduction

Cancer remains the leading cause of death worldwide, with approximately 14.1 million new cancer cases and 8.2 million deaths occurring in 2012 according to the GLOBOCAN estimates [1]. It has been estimated that about one-third of cancers are attributable to diet and lifestyle [2], and a number of studies have reported a relationship between folate intake and cancer risk [3-5].

Folate plays an important role in one-carbon metabolism, and acts as a coenzyme in DNA methylation and synthesis [6]. Folate can provide the methyl group donor S-adenosylmethionine for many biological reactions. It also plays a critical role in the de novo synthesis of purines and thymidylate, which
are necessary for DNA replication and repair [7]. Abnormal folate metabolism can lead to the aberrant distribution of methyl groups and affect DNA biosynthesis and methylation, which is considered as a mechanism in the development of cancer [8].

Methionine synthase reductase (MTRR) is one of the key regulatory enzymes involved in the folate metabolic pathway. It can catalyze the regeneration of methyl cobalamin, which is a cofactor of methionine synthase (MTR) in the remethylation of homocysteine to methionine [9]. Because MTRR plays a vital role in maintaining the active state of MTR, genetic variation within the MTRR gene may be associated with cancer susceptibility. The MTRR gene is located on chromosome 5 at 5p15.2-p15.3, and the most common polymorphism is the substitution of isoleucine with methionine at position 22 (A66G; rs1801394). It has been suggested that the 66GG genotype is negatively correlated with plasma homocysteine levels [10]. A large number of studies have investigated the role of the MTRR A66G polymorphism and cancer risk [11-82], but the results remain controversial. Therefore, we conducted this updated meta-analysis from all eligible studies to derive a more precise estimation of this association.

Materials and methods

Search strategy

A comprehensive literature search was carried out in PubMed, Embase, and Chinese Biomedical (CBM) databases for all relevant articles using the following search terms: “MTRR or methionine synthase reductase or one-carbon metabolism”, “polymorphism or variant or variation” and “cancer or tumor or carcinoma or neoplasm” (the last search was updated on October 21, 2016). Review articles and references cited in the searched studies were examined manually to identify additional relevant articles. Only the most recent study or the one with most participants was included in the final meta-analysis if two or more studies overlapped.

Inclusion and exclusion criteria

The included studies met the following criteria: (1) case-control study design; (2) investigating the association between the MTRR A66G polymorphism and cancer risk; (3) providing detail information for calculating pooled odds ratios (ORs) and their 95% confidence intervals (CIs). Studies were excluded if one of the following existed: (1) not a case-control study; (2) duplicate publications; (3) without detail genotype frequencies; and (4) genotype frequencies in the controls departed from Hardy-Weinberg equilibrium (HWE).

Data extraction

Information was extracted from all eligible studies independently by two authors (Ping Wang and Meilin Wang) according to the inclusion and exclusion criteria listed above. Disagreement was resolved by discussion until consensus was reached. The following information was collected from each study: first author’s surname, year of publication, country of origin, ethnicity, cancer type, control source (hospital-based or population-based), genotyping methods, and numbers of cases and controls with the AA, AG and GG genotypes. Ethnicities were categorized as Asians, Caucasians, Africans or Mixed, which included individuals belonging to more than one ethnic group.

Quality assessment

Quality assessment was performed by two authors independently according to the criteria as described previously [83]. Quality scores of studies ranged from 0 (lowest) to 15 (highest), and the studies were categorized into high quality (scores > 9) and low quality (scores ≤ 9).

Statistical analysis

The strength of association between the MTRR A66G polymorphism and cancer risk was assessed by calculating the ORs with the corresponding 95% CIs. The pooled ORs of 5 comparison models were calculated: homozygous model (GG vs. AA), heterozygous model (AG vs. AA), recessive model (GG vs. (AA + AG)), dominant model [(GG +AG) vs. AA] as well as an allele comparison (G vs. A). The Chi square-based Q-test was used to check heterogeneity between studies. A P value greater than 0.1 for the Q-test indicated the homogeneity among studies, in which case the fixed-effects model (the Mantel-Haenszel method) [84] was adopted. Otherwise, the random-effects model (the DerSimonian and Laird method) [85] was applied. Data were stratified by cancer type (if one cancer type was represented by fewer than two studies, it was merged into the “other cancers” group), ethnicity (Asians, Caucasians, Africans or Mixed), source of control (hospital-based studies and population-based studies), and quality scores (≤ 9 and > 9). Potential publication bias was estimated using Begg’s funnel plot [86] and Egger’s linear regression test [87]. Sensitivity analysis was carried out to evaluate the effect of each individual study on the pooled ORs by excluding studies one-by-one and recalculating the ORs and 95% CIs.

For significant results found in the present meta-analysis, the false-positive report probability (FPRP) was used to evaluate positive associations. We
calculated FPRP with 0.2 as a threshold and assigned a prior probability of 0.1 to detect an OR of 0.67/1.50 (protective/risk effects) for an association with genotypes under investigation. FPRP values < 0.2 were considered as noteworthy associations [88]. All the statistical tests were performed with STATA version 12.0 (Stata Corporation, College Station, TX). All the P values were two-sided, and P < 0.05 was considered statistically significant.

Results

Study characteristics

As shown in Figure 1, a total of 381 published records were identified from PubMed, Embase and CBM by using the search terms described above. By checking the reference lists, we identified 29 additional publications. After screening the abstracts and texts, only 96 publications met the crude inclusion criteria and were selected for further assessment. Among them, five were excluded for containing survival data only [89-93], seven lacked detailed data for further analysis [94-100], eleven deviated from HWE [101-111] and one was a case-only study [112]. Ultimately, 72 publications [11-82] were included in the final meta-analysis (Table 1).

Table 1. Characteristics of studies included in the meta-analysis.

| Surname [ref] | Year | Country | Ethnicity | Cancer type | Control method | Genotype method | Case | Control | MAF  | HWE | Score |
|---------------|------|---------|-----------|-------------|----------------|----------------|------|---------|------|-----|-------|
| Le Marchand [11] | 2002 | USA | Asian | Colorectal | PCR-RFLP | AA 148, AG 81, GG 26 | AA 193, AG 40, GG 39 | 0.29 | 0.374 | 11 |
| Le Marchand [11] | 2002 | USA | Caucasian | Colorectal | PCR-RFLP | AA 26, AG 81, GG 26 | AA 45, AG 6, GG 39 | 0.48 | 0.865 | 10 |
| Le Marchand [11] | 2002 | USA | Mixed | Colorectal | PCR-RFLP | AA 30, AG 34, GG 12 | AA 40, AG 38, GG 9 | 0.32 | 0.995 | 9 |
| Stolzenberg-Solomon [12] | 2003 | China | Asian | Esophagus | PCR-RFLP | AA 50, AG 63, GG 16 | AA 186, AG 179, GG 33 | 0.31 | 0.268 | 14 |
| Stolzenberg-Solomon [12] | 2003 | China | Asian | Gastric | Real-time PCR | AA 43, AG 37, GG 10 | AA 186, AG 179, GG 33 | 0.31 | 0.268 | 13 |
| Gemmati [13] | 2004 | Italy | Caucasian | ALL | PCR-RFLP | AA 28, AG 58, GG 23 | AA 59, AG 122, GG 76 | 0.47 | 0.457 | 10 |
| Gemmati [13] | 2004 | Italy | Caucasian | NHL | PCR-RFLP | AA 51, AG 106, GG 43 | AA 59, AG 122, GG 76 | 0.47 | 0.457 | 10 |
| Otani [14] | 2005 | Japan | Asian | Colorectal | Taqman | AA 58, AG 44, GG 5 | AA 128, AG 82, GG 14 | 0.25 | 0.858 | 8 |
| Shi [15] | 2005 | USA | Caucasian | Lung | PCR-RFLP | AA 162, AG 503, GG 370 | AA 231, AG 542, GG 375 | 0.44 | 0.168 | 11 |
| Zhang [16] | 2005 | USA | Caucasian | Head and neck | PCR-RFLP | AA 114, AG 376, GG 231 | AA 276, AG 589, GG 369 | 0.46 | 0.161 | 11 |
| Chen [17] | 2006 | China | Asian | Colorectal | PCR-RFLP | AA 32, AG 107, GG 253 | AA 89, AG 253, GG NA | NA | NA | 9 |
| Koushik [18] | 2006 | USA | Mixed | Colorectal | Taqman | AA 82, AG 159, GG 116 | AA 163, AG 399, GG 245 | 0.45 | 0.981 | 14 |

http://www.jcancer.org
| Authors | Year | Country | Tumor Type          | Method     | Case Numbers |
|---------|------|---------|---------------------|------------|--------------|
| Liu [70] | 2013 | USA     | Mixed Colorectal    | PCR-RFLP   | 342          |
| Morita [71] | 2013 | Japan   | Asian Colorectal    | PCR-RFLP   | 342          |
| Tomita [72] | 2013 | Brazil  | Mixed Cervical      | Allele-specific | 70         |
| Lisowska [22] | 2007 | Poland  | Caucasian Breast    | PCR-RFLP   | 388          |
| Moore [23] | 2007 | Spain   | Caucasian Bladder   | Illumina   | 267          |
| Petra [24] | 2007 | Slovenia | Colorectal ALL     | PCR-RFLP   | 15           |
| Suzuki [25] | 2007 | Japan   | Asian Head and neck | PCR-RFLP   | 108          |
| Suzuki [26] | 2007 | Japan   | Asian Lung          | Taqman     | 113          |
| Zhang [27] | 2008 | Poland  | Caucasian Gastric   | Taqman     | 56           |
| Bethke [28] | 2008 | Multi-center | Caucasian Brain  | Illumina   | 534          |
| Gra [29] | 2008 | Russia  | Caucasian ALL       | PCR-based bioclp | 109          |
| Gra [29] | 2008 | Russia  | Caucasian AML       | PCR-based bioclp | 26          |
| Gra [30] | 2008 | Russia  | Caucasian NHL       | PCR-based bioclp | 16          |
| Gra [30] | 2008 | Russia  | Caucasian CLL       | PCR-based bioclp | 20          |
| Ikeda [31] | 2008 | Japan   | Asian Colorectal    | MassARRAY  | 51           |
| Ikeda [31] | 2008 | Japan   | Asian Gastric       | MassARRAY  | 83           |
| Kim [32] | 2008 | Korea   | Asian NHL           | PCR-RFLP   | 292          |
| Kwak [33] | 2008 | Korea   | Asian Liver         | PCR-RFLP   | 40           |
| Lima [34] | 2008 | Brazil  | Mixed Multiple myeloma | PCR-RFLP   | 32          |
| Marchal [35] | 2008 | Spain   | Caucasian Prostate  | Real-time PCR | 38          |
| Mir [36] | 2008 | India   | Asian Breast        | PCR-RFLP   | 1            |
| Steck [37] | 2008 | USA     | African Colorectal  | Taqman     | 116          |
| Steck [37] | 2008 | USA     | Caucasian Colorectal | Taqman     | 53           |
| Suzuki [38] | 2008 | Japan   | Asian Breast        | Taqman     | 205          |
| Suzuki [39] | 2008 | Japan   | Asian Pancreatic    | Taqman     | 78           |
| Theodoratou [40] | 2008 | Scotland | Caucasian Colorectal | MassARRAY  | 200          |
| de Jonge [41] | 2009 | Netherlands | Caucasian ALL      | Real-time PCR | 59          |
| Kim [42] | 2009 | Korea   | Asian ALL           | PCR-RFLP   | 38           |
| Kim [42] | 2009 | Korea   | Asian AML           | PCR-RFLP   | 195          |
| Kim [42] | 2009 | Korea   | Asian CML           | PCR-RFLP   | 73           |
| Rouissi [43] | 2009 | Tunisia | African Bladder     | PCR-RFLP   | 59           |
| Burcos [44] | 2010 | Romania | Caucasian Breast    | PCR-RFLP   | 0            |
| Burcos [44] | 2010 | Romania | Caucasian Colorectal | PCR-RFLP   | 11          |
| Cai [45] | 2010 | China   | Asian Prostate      | PCR-RFLP   | 111          |
| Eussen [46] | 2010 | Multi-center | Caucasian Gastric  | MALDI-TOF MS | 58          |
| Sangrajan [47] | 2010 | Thailand | Asian Breast        | Taqman     | 295          |
| Tong [48] | 2010 | Korea   | Asian Cervical      | Multiplexed PCR | 137         |
| Wettergren [49] | 2010 | Sweden  | Colorectal          | Real-time PCR | 22          |
| Curtis [50] | 2011 | USA     | Mixed Colorectal    | Illumina   | 193          |
| Guimaraes [51] | 2011 | Brazil  | Mixed Colorectal    | PCR-RFLP   | 26           |
| Jokic [52] | 2011 | Croatia | Caucasian Colorectal | Taqman     | 53           |
| Metayer [53] | 2011 | USA     | Mixed ALL           | Illumina   | 133          |
| Mostowska [54] | 2011 | Poland  | Caucasian Cervical  | HRM        | 44           |
| Pardini [55] | 2011 | Czech   | Caucasian Colorectal | Taqman     | 113          |
| te Winkel [56] | 2011 | Netherlands | Caucasian ALL  | Real-time PCR | 17          |
| Webb [57] | 2011 | Australia | Mixed Ovarian     | MassARRAY  | 584          |
| Weiner [58] | 2011 | Russia  | Caucasian NHL       | Real-time PCR | 26          |
| Yang [59] | 2011 | China   | Asian ALL           | Real-time PCR | 180          |
| Amigou [60] | 2012 | France  | Caucasian ALL       | Illumina   | 112          |
| Galbiatti [61] | 2012 | Brazil  | Mixed Head and neck | Real-time PCR | 69          |
| Lajin [62] | 2012 | Syria   | Caucasian Breast    | ARMS-PCR   | 40           |
| Pavlik [63] | 2012 | Poland  | Mixed Ovarian       | HRM        | 47           |
| Weber [64] | 2012 | Russia  | Caucasian Breast    | Real-time PCR | 162          |
| Yoo [65] | 2012 | Korea   | Asian Gastric       | MassARRAY  | 655          |
| Yoshimitsu [66] | 2012 | Japan   | Asian Colorectal    | PCR-RFLP   | 281          |
| Yuan [67] | 2012 | China   | Asian Gastric       | MassARRAY  | 27           |
| Chen [68] | 2013 | China   | Asian Cervical      | PCR-RFLP   | 50           |
| Jackson [69] | 2013 | Jamaica | African Prostate    | Taqman     | 111          |
| Liu [70] | 2013 | USA     | Mixed Colorectal    | Illumina   | 264          |
| Morita [71] | 2013 | Japan   | Asian Colorectal    | PCR-RFLP   | 342          |
| Tomita [72] | 2013 | Brazil  | Mixed Cervical      | 70          |
Controls were matched for age, sex and ethnicity in considered as high quality (quality score > 9). Population-based and 33 were hospital-based. Of all the studies, 52 were than two studies. There were 37 studies on Asians, 32 on Africans. Of all the studies, 52 were on Asians, 32 on Africans. Of all the studies, 52 were non-Hodgkin lymphoma (NHL) [13, 30, 32, 58, 78], four each on cervical cancer [48, 54, 68, 72] and liver cancer [33, 74, 75, 81], three each on prostate cancer [35, 45, 69], head and neck cancer [16, 25, 61] and brain cancer [28, 73, 77], and “other cancers” with no more than two studies. There were 37 studies on Asians, 32 studies on Caucasians, 13 studies on mixed ethnicities and three on Africans. Of all the studies, 52 were population-based and 33 were hospital-based. Furthermore, 37 studies were considered as low quality (quality score ≤ 9), and 48 studies (56.5%) were considered as high quality (quality score > 9). Controls were matched for age, sex and ethnicity in most studies.

Meta-analysis results

The main results of the meta-analysis are shown in Table 2 and Figure 2. Pooled analysis indicated a significant association between the MTRR A66G polymorphism and cancer risk (homozygous: OR = 1.08, 95% CI = 1.02-1.15, P = 0.009; recessive: OR = 1.06, 95% CI = 1.00-1.12, P < 0.001 and allele comparison: OR = 1.03, 95% CI = 1.00-1.06, P < 0.001). In the subgroup analysis, statistically significant associations were found for head and neck cancer (homozygous: OR = 1.49, 95% CI = 1.17-1.89, P = 0.076; dominant: OR = 1.30, 95% CI = 1.03-1.64, P = 0.143 and allele comparison: OR = 1.17, 95% CI = 1.04-1.31, P = 0.560), Caucasians (homozygous: OR = 1.09, 95% CI = 1.00-1.19, P = 0.077; dominant: OR = 1.08, 95% CI = 1.00-1.17, P = 0.045 and allele comparison: OR = 1.05, 95% CI = 1.01-1.09, P = 0.193), Africans (homozygous: OR = 1.52, 95% CI = 1.00-2.32, P = 0.577 and allele comparison: OR = 1.23, 95% CI = 1.01-1.49, P = 0.474) and high quality studies (homozygous: OR = 1.07, 95% CI = 1.00-1.15, P = 0.005 and recessive: OR = 1.06, 95% CI = 1.01-1.11, P = 0.262).

Heterogeneity and sensitivity analysis

Substantial heterogeneity was detected among all studies of the MTRR A66G polymorphism and overall cancer risk (homozygous: P = 0.009; heterozygous: P = 0.007; dominant: P = 0.001; recessive: P < 0.001 and allele comparison: P < 0.001). Therefore, the random-effects model was applied to generate wider CIs. Leave-one-out sensitivity analysis was performed and the results suggested the pooled ORs were not influenced by omitting any single study (data not shown).

Publication bias

As shown by the relative symmetric funnel plot (Figure 3) and Egger’s test, no evidence of publication bias was found in the current analysis under any of the models (homozygous: P = 0.913; heterozygous: P = 0.551; dominant: P = 0.510; recessive: P = 0.666 and allele comparison: P = 0.560).

Of the 72 publications, two publications [11, 37] with different ethnic groups were separated as five independent studies and eight publications [12, 13, 29-31, 42, 44, 74] with different cancer types were also treated as 18 independent studies. For those studies [12, 13, 21, 25, 26, 29, 30, 32, 38, 39, 42, 50, 54, 63, 70, 74] with the same control group, the control numbers were calculated once in the total number. Overall, 72 publications including 85 studies of 32,272 cases and 37,427 controls were included in the final meta-analysis. Of the 85 studies, 20 studies focused on colorectal cancer [11, 14, 17, 18, 20, 31, 37, 40, 44, 49-52, 55, 66, 70, 71], ten on breast cancer [19, 22, 36, 38, 44, 47, 62, 64, 76, 82], nine on acute lymphoblastic leukemia (ALL) [13, 24, 29, 41, 42, 53, 56, 59, 60], eight on gastric cancer [12, 27, 31, 46, 65, 67, 74, 79], five on non-Hodgkin lymphoma (NHL) [13, 30, 32, 58, 78], four each on cervical cancer [48, 54, 68, 72] and liver cancer [33, 74, 75, 81], three each on prostate cancer [35, 45, 69], head and neck cancer [16, 25, 61] and brain cancer [28, 73, 77], and “other cancers” with no more than two studies. There were 37 studies on Asians, 32 studies on Caucasians, 13 studies on mixed ethnicities and three on Africans. Of all the studies, 52 were population-based and 33 were hospital-based. Furthermore, 37 studies were considered as low quality (quality score ≤ 9), and 48 studies (56.5%) were considered as high quality (quality score > 9). Controls were matched for age, sex and ethnicity in most studies.

| Year | Country | Ethnicity | Site | Method | PCR | MTRR A66G Polymorphism | OR (95% CI) | P Value |
|------|---------|-----------|------|--------|-----|-----------------------|------------|---------|
| 2013 | China   | Asian     | Brain | Taqman | PCR-RFLP | 209 269 122 225 282 93 | 0.39 0.765 | 12      |
| 2014 | China   | Asian     | Gastric | Taqman | PCR-RFLP | 119 63 9 204 149 25 | 0.26 0.752 | 12      |
| 2014 | China   | Asian     | Liver  | Taqman | PCR-RFLP | 114 64 13 204 149 25 | 0.26 0.752 | 11      |
| 2014 | China   | Asian     | Esophagus | Taqman | PCR-RFLP | 117 74 10 204 149 25 | 0.26 0.752 | 12      |
| 2014 | China   | Asian     | Liver  | Taqman | PCR-RFLP | 103 86 16 112 73 15 | 0.26 0.520 | 6       |
| 2015 | USA     | Caucasian | Breast | Illumina | PCR-RFLP | 158 318 140 165 321 138 | 0.48 0.442 | 14      |
| 2015 | Australia | Mixed    | Brain  | MassARRAY | PCR-RFLP | 80 148 90 102 264 175 | 0.43 0.890 | 11      |
| 2015 | Multi-center | Asian | NHL | MassARRAY | PCR-RFLP | 178 153 41 353 306 63 | 0.30 0.774 | 10      |
| 2016 | Korea   | Asian     | Gastric | Affymetrix | PCR-RFLP | 136 111 23 295 211 35 | 0.26 0.739 | 10      |
| 2016 | Japan   | Pancreatic | Breast | Dynamic Array | PCR-RFLP | 167 157 36 206 158 36 | 0.29 0.473 | 11      |
| 2016 | Brazil  | Mixed     | Liver  | Real-time PCR | PCR-RFLP | 12 50 9 105 179 72 | 0.45 0.787 | 8       |

MAF, minor allele frequency; HB: hospital-based; PB: population-based; NA, not applicable; PCR-RFLP: polymorphism chain reaction restriction fragment length polymorphism; MALDI-TOF MS: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; HRM: high resolution melt; ARMS-PCR: amplification refractory mutation system-PCR; ALL: acute lymphoblastic leukemia; NHL: non-Hodgkin’s lymphoma; AML: acute myelogenous leukemia; CML: chronic myelogenous leukemia; CLL: chronic lymphocytic leukemia.

* Chen [17], Calbiatti [61] and Jackson [69] were only calculated for the dominant model.
* Gra [29] and Tong [48] were only calculated for the recessive model.
* Mir [36] and Burcos [44] (breast cancer) were only calculated for the recessive model and allele comparison, and the number of AA genotype was zero.
FPRP test results

The significant associations were investigated using the FPRP test and the results were shown in Table 3. For a prior probability of 0.1, the FPRP value was 0.128 for the MTRR A66G polymorphism with an increased cancer risk under the homozygous model, and positive associations were also found in head and neck cancer (homozygous: FPRP = 0.017 and allele comparison: FPRP = 0.055), Caucasians (allele comparison: FPRP = 0.087) and high score studies (recessive: FPRP = 0.106). However, no positive association was found between the MTRR A66G polymorphism and cancer risk in Africans.

| Variables       | No. of studies | Sample size (case/controls) | Homozygous  | Heterozygous | Recessive  | Dominant  | Allele comparison |
|-----------------|----------------|----------------------------|-------------|--------------|------------|-----------|-------------------|
|                 |                |                            | OR (95% CI) | OR (95% CI)  | OR (95% CI)| OR (95% CI)| OR (95% CI)       |
| All             | 85             | 32,272/37,427              | 1.08 (1.02-1.15) | 0.009 (0.97-1.06) | 0.007 (1.00-1.12) | <0.001 (1.00-1.06) | <0.001 |
| Cancer type     |                |                            |             |              |            |           |                   |
| Colorectal      | 20             | 8,057/10,465               | 1.09 (0.96-1.25) | 0.051 (0.95-1.16) | 0.030 (0.97-1.11) | 0.462 (0.97-1.19) | 0.006 (1.05-1.12) | 0.007 |
| Breast          | 10             | 6,048/5,872                | 1.08 (0.96-1.21) | 0.488 (0.89-1.11) | 0.131 (0.98-1.22) | 0.001 (0.94-1.11) | 0.362 (1.01-2.11) | 0.018 |
| ALL             | 9              | 1,893/3,770                | 0.90 (0.72-1.13) | 0.228 (0.76-1.03) | 0.367 (0.89-1.14) | 0.013 (0.78-1.02) | 0.472 (0.93-1.05) | 0.547 |
| Gastric         | 8              | 2,756/2,504                | 0.96 (0.72-1.29) | 0.054 (0.80-1.12) | 0.159 (0.82-1.27) | 0.109 (0.78-1.14) | 0.041 (0.97-1.84) | 0.010 |
| NHL             | 5              | 1,357/1,674                | 1.00 (0.74-1.35) | 0.126 (0.84-1.11) | 0.998 (0.74-1.33) | 0.053 (0.89-1.13) | 0.911 (0.89-1.11) | 0.295 |
| Cervical        | 4              | 579/805                    | 1.22 (0.80-1.86) | 0.968 (0.78-1.46) | 0.011 (0.85-1.45) | 0.335 (0.89-1.14) | 0.292 (1.08-1.43) | 0.478 |
| Liver           | 4              | 561/757                    | 1.19 (0.79-1.78) | 0.600 (0.84-2.10) | 0.011 (0.65-1.45) | 0.335 (0.89-1.14) | 1.29 (0.86-1.94) | 0.022 |
| Brain           | 3              | 2,554/2,789                | 1.05 (0.72-1.52) | 0.099 (0.79-1.21) | 0.187 (0.84-1.40) | 0.234 (0.77-1.27) | 0.011 (0.88-1.38) | 0.151 |
| Head and neck   | 3              | 1,223/1,700                | 1.49 (1.17-1.89) | 0.768 (0.79-1.94) | 0.025 (1.15-1.38) | 0.346 (1.03-1.64) | 0.143 (1.06-1.1) | 0.560 |
| Prostate        | 3              | 594/627                    | 1.05 (0.65-1.71) | 0.798 (0.82-1.52) | 0.260 (0.64-1.44) | 0.689 (1.07-1.40) | 0.099 (1.04-1.27) | 0.718 |
| Other cancers   | 16             | 6,650/6,464                | 1.14 (1.01-1.28) | 0.282 (0.94-1.10) | 0.011 (0.91-1.10) | 0.533 (1.00-1.11) | 0.211 (1.06-1.00) | 0.340 |
| Ethnicity       |                |                            |             |              |            |           |                   |
| Asian           | 37             | 11,829/13,248              | 1.11 (0.98-1.24) | 0.080 (0.92-1.05) | 0.063 (0.97-1.22) | 0.006 (1.01-1.05) | 0.057 (1.02-1.98) | 0.001 |
| Caucasian       | 32             | 13,351/16,506              | 1.09 (1.00-1.19) | 0.077 (0.99-1.16) | 0.008 (1.03-1.09) | 0.144 (1.08-1.10) | 0.074 (1.04-1.05) | 0.193 |
| African         | 3              | 619/716                    | 1.52 (1.00-2.32) | 0.577 (0.92-1.60) | 0.553 (1.36-2.02) | 0.751 (1.21-1.51) | 0.624 (1.23-1.49) | 0.474 |
| Mixed           | 13             | 6,473/6,957                | 1.01 (0.88-1.15) | 0.084 (0.86-1.06) | 0.184 (1.12-1.32) | <0.001 (1.00-1.10) | 0.075 (1.01-0.94) | 0.088 |
| Source of control |           |                            |             |              |            |           |                   |
| PB              | 52             | 21,300/24,134              | 1.06 (0.99-1.14) | 0.087 (0.94-1.04) | 0.304 (0.99-1.11) | 0.037 (1.01-1.06) | 0.135 (1.02-0.99) | 0.075 |
| HB              | 33             | 10,972/13,293              | 1.12 (0.99-1.26) | 0.019 (0.97-1.16) | 0.002 (1.07-1.41) | <0.001 (1.08-1.18) | 0.001 (1.04-0.86) | <0.001 |
| Score           |                |                            |             |              |            |           |                   |
| Low             | 37             | 6,610/9,768                | 1.13 (0.99-1.29) | 0.265 (0.96-1.16) | 0.144 (0.90-1.24) | 0.000 (0.98-1.17) | 0.299 (1.05-0.98) | 0.042 |
| High            | 48             | 25,662/27,659              | 1.07 (1.00-1.15) | 0.005 (0.95-1.05) | 0.010 (1.06-1.1) | 0.262 (1.07-1.08) | <0.001 (1.02-0.99) | 0.001 |

Het, heterogeneity; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin’s lymphoma; PB, population based; HB, hospital based.

* The number of controls was only calculated once if the same controls were used.
Figure 2. Forest plot for overall cancer risk associated with the MTRR A66G polymorphism by a recessive model. For each study, the estimated OR and its 95% CI are plotted with a box and a horizontal line. △, pooled ORs and its 95% CIs.
Figure 3. Funnel plot for the MTRR A66G polymorphism and cancer risk by a recessive model.

### Table 3. False-positive report probability values for associations between cancer risk and genotypes of MTRR A66G polymorphism.

| Genotype                          | Crude OR (95% CI) | P-value * | Statistical Power * | Prior probability | 0.25 | 0.1 | 0.01 | 0.001 | 0.0001 |
|-----------------------------------|-------------------|-----------|--------------------|------------------|------|-----|------|------|--------|
| All patients                      |                   |           |                    |                  |      |     |      |      |        |
| Homozygous                        | 1.08 (1.02-1.15)  | 0.016     | 1.00               | 0.047            | 0.128| 0.618| 0.942| 0.994|
| Recessive                         | 1.06 (1.00-1.12)  | 0.038     | 1.00               | 0.102            | 0.255| 0.790| 0.974| 0.997|
| Allele comparison                 | 1.03 (1.00-1.06)  | 0.044     | 1.00               | 0.116            | 0.282| 0.812| 0.978| 0.998|
| Cancer type-head and neck cancer  |                   |           |                    |                  |      |     |      |      |        |
| Homozygous                        | 1.49 (1.17-1.89)  | 0.001     | 0.522              | 0.006            | 0.017| 0.161| 0.660| 0.951|
| Dominant                          | 1.30 (1.03-1.64)  | 0.027     | 0.886              | 0.083            | 0.214| 0.750| 0.968| 0.997|
| Allele comparison                 | 1.17 (1.04-1.31)  | 0.006     | 1.00               | 0.019            | 0.055| 0.391| 0.886| 0.985|
| Ethnicity-Caucasian               |                   |           |                    |                  |      |     |      |      |        |
| Homozygous                        | 1.09 (1.00-1.19)  | 0.054     | 1.00               | 0.140            | 0.328| 0.843| 0.982| 0.998|
| Dominant                          | 1.08 (1.00-1.17)  | 0.059     | 1.00               | 0.151            | 0.349| 0.885| 0.983| 0.998|
| Allele comparison                 | 1.05 (1.01-1.09)  | 0.010     | 1.00               | 0.031            | 0.087| 0.511| 0.913| 0.991|
| Ethnicity-African                 |                   |           |                    |                  |      |     |      |      |        |
| Homozygous                        | 1.52 (1.02-2.32)  | 0.052     | 0.476              | 0.248            | 0.497| 0.916| 0.991| 0.999|
| Allele comparison                 | 1.23 (1.01-1.49)  | 0.034     | 0.979              | 0.095            | 0.240| 0.777| 0.972| 0.997|
| Score-high                        |                   |           |                    |                  |      |     |      |      |        |
| Homozygous                        | 1.07 (1.00-1.15)  | 0.066     | 1.00               | 0.165            | 0.372| 0.867| 0.985| 0.998|
| Recessive                         | 1.06 (1.01-1.11)  | 0.013     | 1.00               | 0.038            | 0.106| 0.567| 0.930| 0.992|

*Chi-square test was used to calculate the genotype frequency distributions.

*Statistical power was calculated using the number of observations in the subgroup and the OR and P values in this table.

**Discussion**

Folate is a critical coenzyme in DNA synthesis, and the maintenance of methylation, and folate deficiency has been reported to be associated with various human malignancies [113, 114]. MTRR plays a key role in folate-dependent homocysteine remethylation and is required in the regulation of MTR activity. The A66G polymorphism is one of the most common polymorphisms in the MTRR gene, which was first reported in 1998 [115], and the variant enzyme has reduced affinity for MTR [116]. The reported associations between the MTRR A66G polymorphism and cancer susceptibility are inconsistent due to the small sample sizes in individual studies, ethnic differences and research methodology.

Our present study represents an updated comprehensive meta-analysis of the association between the MTRR A66G polymorphism and cancer risk and included 85 studies with 32,272 cases and 37,427 controls. The results revealed that the MTRR A66G polymorphism was significantly associated
with an increased overall cancer risk. In the subgroup analysis, the association was more evident for head and neck cancer, Caucasians, Africans and high quality studies. However, the results for Africans need further validation due to the high probability of false-positive reports. Furthermore, no potential publication bias was detected by the funnel plot and Egger’s regression test, indicating the robustness of the results in this study.

One previous meta-analysis focused on the MTRR A66G polymorphism and overall cancer risk. In the meta-analysis by Han et al. [117], which included 35 studies with 18,661 cases and 27,678 controls, an increased overall cancer risk was observed only under the allele comparison and homozygous model. In the subgroup analysis, significantly increased risks were found in Asians. We found this polymorphism to be associated with an increased overall risk also under the recessive model and increased cancer risks in head and neck cancer, Caucasians and Africans, but not in Asians, which were different from the previous meta-analysis; this result presumably occurred because our analysis was based on a much larger sample size, thereby increasing the statistical power. In the subgroup analysis by cancer type, we did not find any significant association between the MTRR A66G polymorphism and colorectal cancer in any comparison models, a finding that was inconsistent with previous meta-analyses [6, 118]. The discrepancy occurred because, in the current study, we added many newly published studies and even included several Chinese publications, allowing the more precise detection of an association.

Large and well-designed studies with “statistically significant” results for genetic variants turned out to be false-positive findings [119, 120]. Thus, we used the FPRP test to investigate positive associations in the current meta-analysis. Interestingly, the FPRP test results showed that the MTRR A66G polymorphism could actually increase cancer susceptibility. In the subgroup analysis, the FPRP test indicated that the MTRR A66G polymorphism increased cancer susceptibility in head and neck cancer, Caucasians and high score studies. The significant association with Africans in the present meta-analysis was false positive, which may due to the limited sample size.

Although we conducted a comprehensive literature search and included the latest studies on the MTRR A66G polymorphism and cancer risk, some possible limitations in this meta-analysis should be addressed. First, the number of cases in the individual studies was small (<1000) in all but eight studies [15, 19, 22, 23, 28, 57, 65, 70]; this limitation may affect the investigation of the real association. Second, our results were based on unadjusted estimates, so the estimates were relatively imprecise. Third, the effects of gene-gene, and gene-environment interactions were not evaluated due to the lack of original data, which may affect cancer risk. Fourth, in the subgroup analysis, only three studies were carried out in Africans, which may lead to relatively weak power to detect the real association. Finally, only studies published in English and Chinese were included, so we may have missed publications in other languages.

In conclusion, we performed this updated meta-analysis with the latest published studies and obtained a more precise estimation of the association between the MTRR A66G polymorphism and cancer risk. However, it is necessary to conduct well-designed prospective studies with larger sample sizes to verify our findings.

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Competing Interests

The authors have declared that no competing interest exists.

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Chen K, Song L, Jin MJ, Fan CH, Jiang QT, Yu WP. [Association between genetic polymorphisms in folate metabolic enzyme genes and colorectal cancer: a nested case-control study]. Zhonghua Zhong Liu Za Zhi. 2008; 28: 429-432.

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