RESEARCH ARTICLE

Geographical and Ethnic Distributions of the MTHFR C677T, A1298C and MTRR A66G Gene Polymorphisms in Chinese Populations: A Meta-Analysis

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Abstract

Background

The geographical and ethnic distributions of the polymorphic methylenetetrahydrofolate reductase (MTHFR) mutations (C677T and A1298C) and methionine synthase reductase (MTRR) mutation (A66G) remain heterogeneous in China. The goal of this study was to estimate the pooled frequencies of the alleles and associated genotypes of these gene polymorphisms among healthy populations in Mainland China.

Objective and Methods

We systematically reviewed published epidemiological studies on the distributions of 3 genetic variants in Chinese healthy populations living in Mainland China through a meta-analysis. The relevant electronic databases were searched. All of the raw data of the eligible citations were extracted. The frequency estimates were stratified by geography, ethnicity and sex.

Results

Sixty-six studies were identified with a total of 92277 study participants. The meta-analysis revealed that the frequencies of the MTHFR C677T, A1298C, and MTRR A66G gene polymorphisms varied significantly between different ethnic groups and along geographical gradients. The frequencies of the 677T allele and 677TT genotype increased along the southern-central-northern direction across Mainland China (all P values < 0.001). The frequencies of the 1298C, 1298CC, 66G and 66GG genotypes decreased along the southern-central-northern direction across the country (all P values < 0.001).
Conclusions

Our meta-analysis strongly indicates significant geographical and ethnic variations in the frequencies of the C677T, A1298C, and A66G gene polymorphisms in the folate metabolism pathway among Chinese populations.

Introduction

Multiple epidemiological studies have demonstrated that homocysteine is an important biomarker with biological functions in the folate metabolism pathway. Hyperhomocysteinemia (HHCY) is a medical health problem characterized by elevated homocysteine concentrations in the plasma that has been identified as a key pathophysiological risk factor for a series of adverse events, including neural-tube defects, vascular dementia, pregnancy complications, cancers and psychiatric disorders [1–4]. Previous studies have revealed that the regulation of the plasma levels of homocysteine are quite complex and involve both environmental factors (such as folate acid and vitamin B12 intake) and hereditary components [5]. However, how a number of genes and hereditary determinants might contribute to HHCY remains unclear. Mutations in some key genes encoding homocysteine-metabolizing enzymes, such as methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C and methionine synthase reductase (MTRR) A66G, may contribute to the risk of the development of hyperhomocysteinemia and thus lead to clinical disorders [6].

The enzyme MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the carbon donor for the methylation of homocysteine to methionine [7]. The C677T polymorphism is a point mutation at position 677 of the MTHFR gene that causes the substitution of alanine with valine, which leads to a reduction in enzyme activity and causes mild to moderate hyperhomocysteinemia and reduces plasma folate levels. Genome-wide association studies (GWASs) have confirmed the association between the MTHFR C677T genotype and homocysteine levels in healthy populations [8]. Along with those investigations, several studies have proposed that double 677CT/1298AC heterozygosity can result in a reduction in enzymatic activity that represents an important risk factor for congenital anomalies, particularly in patients with low blood folate and vitamin B12 concentrations [9].

The frequency distributions of MTHFR and MTRR polymorphisms, especially C677T, vary substantially between different regional and ethnic groups. For example, the frequency of the 677T allele has been found to be highest in north India (16.7%) and lowest was in east India (1.1%). Moreover, the highest frequency of the 677TT genotype has been found in the Rajput population (7.8%), and this genotype is absent in the Kom, Meitei, Paite, Thadou, Kabui, Munda, Oraon and Naikda population groups in India [10]. A number of studies have investigated the C677T and A1298C in MTHFR and A66G in MTRR polymorphisms in different ethnic and geographical regions in Chinese general populations, however, the results have been irreproducible and inconclusive [11].

Accurate information about the geographical and ethnic distributions of the alleles and associated genotypes of MTHFR and MTRR in Mainland China will enable the design of proper interventions (e.g., folic acid supplementation) in the general population to reduce the rates of some medical diseases [12].

We conducted this comprehensive meta-analysis that integrated multiple studies to provide an overall assessment of the key polymorphisms in the major folate pathway genes among
general Chinese populations. Sex-stratified and northern-central-southern gradients in the heterozygosities and allele frequencies were also assessed.

**Materials and Methods**

**Literature database**

The following major electronic literature databases were searched in September 2015 without language restrictions: PubMed, the Chinese National Knowledge Infrastructure (CNKI), the Chinese Wanfang Database, the Chinese VIP Database, and Google Scholar. The keywords and medical subject headings “MTHFR”, “MTRR”, “methylene tetrahydrofolate reductase”, “methionine synthase reductase”, “folate pathway”, “polymorphisms” or “SNP”, and “Chinese” or “China” were used to scan for potentially relevant studies.

**Inclusion/exclusion criteria**

The identified studies were eligible for inclusion if they met the following criteria: (1) published in Chinese or English, (2) the study participants were general Chinese populations who lived in Mainland China, (3) the evaluation of data related to any or all of the polymorphisms in MTHFR or MTRR in general Chinese populations, and (4) included data regarding genotype/allele counts of the C677T, A1298C and A66G polymorphisms among the population for the estimation of the frequencies and 95% confidence intervals (95% CIs). Studies were excluded if they met the following criteria: (1) reviews, lectures, editorials or correspondence letters, (2) the study participants were evaluated in terms of folate pathway gene polymorphisms associated with relevant diseases, (3) duplicated studies were eliminated, and only recently published studies were ultimately selected, and (4) if the same data were published in English and Chinese, only the English-language articles were included.

**Data extraction**

Two authors (XM Wang and JJ Fu) independently extracted the following information: the first author’s name, publication year, investigated location, ethnic groups, age, sample source, sample size, genotyping method, genotype distribution, frequency, and 95% CI.

**Statistical analysis**

The Hardy-Weinberg equilibrium (HWEs) were evaluated to determine whether the MTHFR C677T and A1298C and MTRR A66G genotype distributions were in genetic equilibrium (threshold set to 0.05) [13]. Meta-analyses of the prevalences of the allele frequencies (e.g., C677T: TT vs. total genotypes) and allele contrasts (e.g., C677T: T vs. total C+T) were performed using Stata statistical software version 13.0 (Stata corporation LP, College Station, Texas, USA). A random-effects model was used to account for the pooled relevant genotype frequencies and their corresponding 95% CIs. Stratified analyses were performed according to the northern-central-southern gradient, ethnicity, and sex. The heterogeneity among the studies was evaluated with the Cochrane chi-square ($\chi^2$) and quantified with the $I^2$ statistic [14–15]. Publication bias was evaluated using Begg’s funnel plots and Egger’s test (significant at $P<0.1$) [16–17].

**Results**

**Characteristics of the studies**

In total, 495 articles were identified, of which 471 potentially relevant citations were included for further evaluation. Eventually, 68 articles (66 on the C677T, 51 on the A1298C, and 43 on
the A66G polymorphisms) with a total of 92277 participants met the inclusion criteria [18–85] (Fig 1). The main characteristics of the studies on the MTHFR C677T and A1298C and MTRR A66G polymorphisms are listed in Tables 1–3, respectively. In the majority of the studies, buccal cells were obtained and tested with real-time polymerase chain reaction (RT-PCR); otherwise, blood samples were tested with restriction fragment length polymorphism (RFLP) analysis. The genotype frequencies indicated that all of the polymorphisms were in HWE in all of the samples.

Pooled frequencies of the allele genotypes of the three gene polymorphisms in the Chinese general population

Table 4 illustrates the summarized national estimates of the 677TT and 677T frequencies among healthy populations from 1998 to 2015. Taking all populations together, the frequencies of the 677TT genotype and the 677T allele in the healthy Chinese population were 20% (18%-23%) and 42% (38%-45%), respectively (S1 and S2 Files). Overall, the combined estimated frequencies of the 1298CC genotype and the 1298C allele in the healthy Chinese population were 5% (4%-5%) and 20% (18%-22%), respectively (S3 and S4 Files). The average frequencies of the 66GG genotype and the 66G allele in the healthy Chinese population were 7% (6%-7%) and 26% (25%-28%), respectively (S5 and S6 Files).

Geographical distributions of the three polymorphisms in the folate pathway

The allele and genotype frequencies of the three polymorphisms according to geographical region are given in Table 5. The genotype frequencies of the MTHFR C677T and 677T alleles and the 677TT genotype frequency exhibited increases in the southern-central-northern direction in Mainland China. The frequencies of the 677T allele and the 677TT genotype increased from lower values (5% and 17%, respectively) in Guangxi, to intermediate values (12% and 32%, respectively) in Anhui, to higher values (39% and 62%, respectively) in Shandong. Taken together, the frequencies of the 677TT genotype and the 677T allele along the geographical gradient were 7% (5%-8%) and 25% (23%-27%) in southern, 19% (16%-21%) and 41% (36%-
| Author | Publicationyear | Location | Ethnicgroup | Age | Sample | Sample collection | Sample size(male/female) | Method | Genotype | Tallelic |
|--------|-----------------|----------|-------------|-----|--------|------------------|--------------------------|--------|----------|---------|
| Yu JM  | 2000            | mixed    | mixed       | not given | blood | convenient       | 200                       | RFLP   | 84       | 100     | 16      | 132     |
| Pei LJ | 2000            | mixed    | mixed       | not given | blood | populational-based | 277                      | RFLP   | 97       | 126     | 54      | 234     |
| Zhu HP | 1998            | mixed    | mixed       | not given | blood | convenient       | 117                       | RFLP   | 50       | 50      | 17      | 84      |
| Yang BH| 2001            | Anhui    | Han         | 20–55    | blood | convenient       | 55(30/25)                 | RFLP   | 19       | 21      | 15      | 51      |
| Chen SQ| 2002            | Guangdong| Han         | average 40| blood | convenient       | 143(68/75)               | RFLP   | 90       | 50      | 3       | 56      |
| Sun WP | 2003            | Shanxi   | Han         | 37–78   | blood | populational-based | 96(58/38)                | RFLP   | 26       | 53      | 17      | 87      |
| Shen LP| 2005            | Guangxi  | Han         | 23–34   | blood | convenient       | 200(female)              | RFLP   | 119      | 68      | 13      | 94      |
| Xiao Y | 2005            | Guangxi  | mixed       | 18–22   | blood | populational-based | 317(138/179)             | RFLP   | 221      | 90      | 6       | 102     |
| Zhang CS| 2005           | Shandong | Han         | 44.7±7.5| blood | convenient       | 86(42/44)                | RFLP   | 11       | 42      | 33      | 108     |
| Li AF  | 2007            | Henan    | Han         | 56±8    | blood | populational-based | 500(274/226)            | RFLP   | 163      | 173     | 164     | 501     |
| Dai X  | 2008            | Ningxia  | mixed       | 18–22   | blood | convenient       | 315(124/191)            | RFLP   | 47       | 221     | 47      | 315     |
| Mao FR | 2008            | mixed    | mixed       | not given| blood | populational-based | 1015                     | RFLP   | 430      | 505     | 80      | 665     |
| Chen F | 2009            | Henan    | Han         | 35–76   | blood | convenient       | 495(320/175)            | RFLP   | 181      | 182     | 132     | 446     |
| Shan KR| 2009            | Guizhou  | Miao        | not given| blood | populational-based | 108                      | RFLP   | 88       | 17      | 3       | 23      |
| Chen YX| 2010            | Shanxi   | Han         | 25–35   | blood | not given        | 50(female)               | RFLP   | 6        | 24      | 20      | 64      |
| He XM  | 2010            | mixed    | Han         | not given| blood | populational-based | 1017(female)            | RFLP   | 355      | 422     | 220     | 882     |
| Jiang HO| 2010           | Hunan    | Han         | 20–70   | blood | populational-based | 120                      | RFLP   | 64       | 41      | 15      | 71      |
| Zhang QF| 2010           | Hainan   | mixed       | 19–46   | buccal cells | convenient       | 100(8female)            | RT-PCR | 559      | 310     | 139     | 588     |
| Zhang L | 2010           | Guangxi  | mixed       | not given| blood | populational-based | 1466(723/743)          | RFLP   | 682      | 678     | 106     | 890     |
| Lao HH | 2011            | Hainan   | mixed       | not given| buccal cells | populational-based | 11437(female)        | RT-PCR | 6678     | 3741    | 1018    | 5777    |
| Zhang Y | 2012            | Sichuan  | Han         | not given| buccal cells | populational-based | 2573(female)           | RT-PCR | 1047     | 1171    | 355     | 1881    |
| Wu HZ  | 2011            | Henan    | Han         | 20–35   | blood | populational-based | 78(39/39)               | RFLP   | 38       | 31      | 9       | 49      |
| He YX  | 2012            | Henan    | Han         | 19–44   | buccal cells | convenient       | 109(female)             | RT-PCR | 198      | 493     | 402     | 1297    |
| Yang Y | 2012            | Jiangsu  | Han         | 27.0±4.4| buccal cells | convenient       | 2885(female)           | RT-PCR | 877      | 1378    | 630     | 2638    |
| Cong YY| 2012            | Shandong | Han         | 29.4±7.7| buccal cells | convenient       | 1041(female)           | RT-PCR | 130      | 457     | 454     | 1365    |
| Zhang YL| 2012           | Shandong | Han         | 28.7±5.8| buccal cells | convenient       | 825(female)            | RT-PCR | 138      | 398     | 289     | 976     |
| Chen HB| 2012            | Shanxi   | Han         | not given| blood | convenient       | 63(31/32)              | RT-PCR | 10       | 31      | 22      | 75      |
| Gao LJ | 2012            | Guangdong| Han         | 27.6±4.0| buccal cells | convenient       | 359(female)            | RT-PCR | 186      | 134     | 39      | 212     |
| Du LL  | 2013            | Guangxi  | Zhuang      | 70–104  | blood | populational-based | 973(339/634)          | RT-PCR | 677      | 252     | 44      | 340     |
| Yang BY| 2013            | Guangxi  | mixed       | 18–47   | blood | populational-based | 15357(952/14405)      | RT-PCR | 4939     | 6791    | 3827    | 14045   |
| Wang LN| 2012            | Xinjiang | mixed       | 19–65   | blood | convenient       | 300(144/156)           | RFLP   | 58       | 196     | 46      | 288     |
| Xiu X  | 2013            | Shandong | Han         | 19–40   | buccal cells | convenient       | 2934(female)           | RT-PCR | 442      | 1354    | 1138    | 3630    |
| Chen YX| 2013            | Shanxi   | Han         | 22–73   | blood | convenient       | 192(94/98)             | RFLP   | 32       | 97      | 63      | 223     |
| Wang WP| 2013            | Hubei    | Han         | 28.2±3.3| buccal cells | convenient       | 289(female)            | RT-PCR | 1069     | 1367    | 463     | 2293    |
| Gao H  | 2013            | Hubei    | Han         | 18–53   | buccal cells | convenient       | 1902(female)           | RT-PCR | 696      | 902     | 304     | 1510    |
| Wan LJ | 2013            | Yunnan   | Han         | 27.5±4.0| buccal cells | convenient       | 297(female)            | RT-PCR | 116      | 139     | 42      | 223     |
| Yan ZM | 2013            | Hainan   | Han         | 27.2±5.3| buccal cells | convenient       | 122(female)            | RT-PCR | 756      | 390     | 75      | 540     |
| Zhang T| 2013            | Guizhou  | minority    | mixed   | blood | populational-based | 597(318/279)          | RT-PCR | 398      | 180     | 19      | 218     |
| Author  | Publicationyear | Location | Ethnicgroup | Age   | Sample | Sample collection | Sample size(male/female) | Method | Genotype | Tallelic |
|---------|----------------|----------|-------------|-------|--------|-------------------|--------------------------|--------|----------|----------|
| Huang GX | 2013           | Hainan   | mixed       | mixed | buccal cells | convenient        | 1841(female) | RT-PCR | 1219 548 74 | 696      |
| Luo XL  | 2014           | Hubei    | Han         | 27.3±5.2 | buccal cells | convenient        | 1077(female) | RT-PCR | 429 482 166 | 814      |
| Wang FX | 2014           | Shannxi  | Han         | 22–35  | buccal cells | convenient        | 1508(female) | RT-PCR | 918 249 341 | 931      |
| Hao YY  | 2014           | Xinjiang | mixed       | mixed | buccal cells | convenient        | 210(female) | RT-PCR | 83 86 41 | 168      |
| Yan Q   | 2014           | Shandong | Han         | 28.8±3.4 | buccal cells | convenient        | 2670(female) | RT-PCR | 497 1313 860 | 3033     |
| Xing JF | 2014           | Henan    | Han         | 28.2±4.2 | buccal cells | convenient        | 425(female) | RT-PCR | 57 207 158 | 523      |
| Hu JW   | 2015           | Sichuan  | Han         | 25.4±4.3 | buccal cells | convenient        | 4865(female) | RT-PCR | 1443 1845 675 | 3195     |
| Huang QH | 2015          | Jiangsu  | Han         | 26.5±4.3 | buccal cells | convenient        | 348(female) | RT-PCR | 99 192 58 | 308      |
| Li JH   | 2015           | Hebei    | Han         | 27.3±4.9 | buccal cells | convenient        | 1267(female) | RT-PCR | 220 617 430 | 1477     |
| Xiang CG | 2015          | Sichuan  | Han         | 26.0±4.8 | buccal cells | convenient        | 656(female) | RT-PCR | 238 302 116 | 534      |
| Jiang W | 2014           | Guangxi  | Han         | 28.0±4.5 | buccal cells | convenient        | 948(female) | RT-PCR | 572 315 61 | 437      |
| Ouyang QQ | 2014         | Shandong | Han         | 22–39  | blood    | convenient        | 98(female) | RFLP   | 24 52 22 | 96       |
| Chen XL | 2014           | Guangxi  | Han         | 27.7±4.4 | buccal cells | convenient        | 564(female) | RT-PCR | 82 271 211 | 693      |
| Ma LM   | 2015           | Heilongjiang | Han     | 28.1±5.5 | buccal cells | convenient        | 455(female) | RT-PCR | 78 240 137 | 514      |
| Tang HY | 2014           | Shandong | Han         | 27.7±3.8 | buccal cells | convenient        | 787(female) | RT-PCR | 107 373 307 | 987      |
| Tian Y  | 2014           | Jiangsu  | Han         | 27.0±4.8 | buccal cells | convenient        | 524(female) | RT-PCR | 185 240 99 | 438      |
| Lu GR   | 2014           | Shandong | Han         | 28.5±5.0 | buccal cells | convenient        | 1352(female) | RT-PCR | 201 625 526 | 1677     |
| Jiao FY | 2014           | Shandong | Han         | 28.2±4.2 | buccal cells | convenient        | 529(female) | RT-PCR | 93 261 175 | 611      |
| Gao X   | 2014           | Hebei    | Han         | 28.3±4.3 | buccal cells | convenient        | 860(female) | RT-PCR | 158 429 273 | 829      |
| Luo SQ  | 2015           | Guangxi  | Miao        | not given | buccal cells | convenient        | 818(female) | RT-PCR | 593 208 12 | 242      |
| Yu YH   | 2015           | Jilin    | Han         | 28.5±4.3 | buccal cells | convenient        | 2620(female) | RT-PCR | 551 1253 816 | 2885     |
| Li XX   | 2015           | Jiangsu  | Han         | 26.7±3.6 | buccal cells | convenient        | 4008(female) | RT-PCR | 1290 1984 734 | 1431     |
| Wang SY | 2015           | Hunan    | Han         | 26.7±4.6 | buccal cells | convenient        | 1701(female) | RT-PCR | 725 762 214 | 1190     |
| Wu WQ   | 2015           | Jiangsu  | Han         | 26.4±4.5 | buccal cells | convenient        | 644(female) | RT-PCR | 189 308 147 | 602      |
| Mao WC  | 2015           | Guizhou  | mixed       | not given | buccal cells | convenient        | 1232(female) | RT-PCR | 468 416 158 | 832      |
| Cui HL  | 2015           | Henan    | Han         | 28.9±4.7 | buccal cells | convenient        | 1253(female) | RT-PCR | 201 542 510 | 1562     |
| Liu XL  | 2014           | Ningxia  | Han         | 29.4±5.3 | buccal cells | convenient        | 443(female) | RT-PCR | 113 228 102 | 432      |

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Table 1. (Continued)
| Author | Publication year | Location | Ethnic group | Age | Sample | Sample collection | Sample size (male/female) | Method | Genotype | C allelic |
|--------|-----------------|----------|--------------|-----|--------|------------------|--------------------------|--------|----------|----------|
| Zhu HP | 1998            | mixed    | mixed        | not given | blood | convenient      | 117                       | RFLP   | AA 69 | AC 41 | CC 7 | 55 |
| Sun WP | 2003            | Shannxi  | Han          | 37–78 | blood | population-based | 96(58/38)                 | RFLP   | AA 61 | AC 32 | CC 3 | 38 |
| Xiao Y | 2005            | Guizhou  | mixed        | not given | blood | population-based | 317(138/179)             | RFLP   | AA 100 | AC 184 | CC 33 | 250 |
| Zhang CS | 2005         | Shandong  | Han          | 44.7±7.5 | blood | convenient | 86(42/44)                 | RFLP   | AA 67 | AC 19 | CC 0 | 19 |
| Mao FR | 2008            | mixed    | mixed        | not given | blood | population-based | 998                       | RFLP   | AA 391 | AC 385 | CC 222 | 829 |
| Chen F | 2009            | Henan    | Han          | 35–76  | blood | convenient      | 495(320/179)             | RFLP   | AA 387 | AC 105 | CC 3 | 111 |
| He XM  | 2010            | mixed    | Han          | not given | blood | population-based | 1017(female)             | RFLP   | AA 649 | AC 322 | CC 46 | 414 |
| Zhang QF | 2010           | Hainan   | mixed        | 19–46  | blood | convenient      | 1008(female)             | RT-PCR | AA 585 | AC 342 | CC 81 | 504 |
| Lao HH | 2011            | Hainan   | not given    | blood | population-based | 11437(female)          | RT-PCR | AA 6481 | AC 4145 | CC 811 | 5767 |
| Zhang Y | 2012            | Sichuan  | Han          | 44.7±7.5 | blood | convenience    | 317                       | RFLP   | AA 1612 | AC 800 | CC 1122 | 166 |
| Wu HZ  | 2011            | Anhui    | Han          | 20–35  | blood | population-based | 78(39/39)                | RFLP   | AA 46 | AC 30 | CC 2 | 34 |
| He YX  | 2012            | Henan    | Han          | 19–44  | blood | convenient      | 1093(female)             | RT-PCR | AA 798 | AC 269 | CC 26 | 321 |
| Yang Y | 2012            | Jiangsu  | Han          | 27.0±4.4 | blood | convenient      | 2885(female)             | RT-PCR | AA 1993 | AC 791 | CC 101 | 993 |
| Cong YY | 2012           | Shandong | Han          | 29.4±7.7 | blood | convenient      | 1041(female)             | RT-PCR | AA 822 | AC 204 | CC 15 | 234 |
| Zhang YL | 2012           | Shandong | Han          | 28.7±5.8 | blood | convenient      | 825(female)              | RT-PCR | AA 627 | AC 178 | CC 20 | 218 |
| Gao LJ | 2012            | Guangdong| Han          | 27.6±4.0 | blood | convenient      | 359(female)              | RT-PCR | AA 221 | AC 112 | CC 26 | 164 |
| Yang BY | 2013            | mixed    | Han          | 18–47  | blood | population-based | 13473                    | RT-PCR | AA 9000 | AC 3944 | CC 529 | 5002 |
| Xiu X  | 2013            | Shandong | Han          | 19–40  | blood | convenient      | 2934(female)             | RT-PCR | AA 2224 | AC 672 | CC 38 | 744 |
| Wang WP | 2013            | Hubei    | Han          | 28.2±3.3 | blood | convenient      | 2899(female)             | RT-PCR | AA 1901 | AC 866 | CC 132 | 1130 |
| Gao H  | 2013            | Hubei    | mixed        | 18–53  | blood | population-based | 1902(female)             | RT-PCR | AA 1283 | AC 558 | CC 61 | 680 |
| Wan LJ | 2013            | Yunnan   | Han          | 27.5±4.0 | blood | convenient      | 297(female)              | RT-PCR | AA 194 | AC 95 | CC 8 | 111 |
| Yan ZM | 2013            | Hainan   | Han          | 27.2±5.3 | blood | convenient      | 1221(female)             | RT-PCR | AA 699 | AC 435 | CC 87 | 609 |
| Zhang T | 2013            | Guizhou  | minority     | mixed   | blood | population-based | 597(318/279)            | RT-PCR | AA 311 | AC 243 | CC 43 | 329 |
| Wang P  | 2013            | Xinjiang | mixed        | Not given | blood | convenient      | 300(female)              | RT-PCR | AA 204 | AC 91 | CC 5 | 101 |
| Huang GX | 2013           | Hainan   | mixed        | blood | convenient      | 1841(female)             | RT-PCR | AA 999 | AC 694 | CC 148 | 990 |
| Luo XL | 2014            | Hubei    | Han          | 27.3±5.2 | blood | convenient      | 1077(female)             | RT-PCR | AA 702 | AC 347 | CC 28 | 403 |
| Wang FX | 2014            | Shanxi   | Han          | 22–35  | blood | convenient      | 1508(female)             | RT-PCR | AA 542 | AC 912 | CC 54 | 1020 |
| Hao YY | 2014            | Xinjiang | mixed        | mixed   | blood | convenient      | 210(female)              | RT-PCR | AA 135 | AC 64 | CC 11 | 86 |
| Yan Q  | 2014            | Shandong | Han          | 28.8±3.4 | blood | convenient      | 2670(female)             | RT-PCR | AA 1936 | AC 685 | CC 49 | 783 |
| Xing JF | 2014            | Henan    | Han          | 28.2±4.2 | blood | convenient      | 425(female)              | RT-PCR | AA 316 | AC 102 | CC 4 | 110 |
| Hu XW  | 2015            | Hubei    | NA           | 28.2±4.2 | blood | convenient      | 3963(female)             | RT-PCR | AA 2661 | AC 1168 | CC 134 | 1436 |
| Jia XP | 2015            | Sichuan  | Han          | 25.4±4.3 | blood | convenient      | 4865(female)             | RT-PCR | AA 3096 | AC 1555 | CC 214 | 1983 |
| Huang QH | 2015         | Jiangsu  | Han          | 26.5±4.3 | blood | convenient      | 348(female)              | RT-PCR | AA 231 | AC 106 | CC 10 | 126 |
| Li JH  | 2015            | Hebei    | Han          | 27.3±4.9 | blood | convenient      | 1267(female)             | RT-PCR | AA 947 | AC 296 | CC 24 | 344 |
| Xiang CG | 2015           | Sichuan  | Han          | 26.0±4.8 | blood | convenient      | 656(female)              | RT-PCR | AA 428 | AC 205 | CC 23 | 251 |
| Jiang W | 2014            | Guangxi  | Han          | 28.0±4.5 | blood | convenient      | 948(female)              | RT-PCR | AA 535 | AC 344 | CC 69 | 482 |
| Chen XL | 2014            | Guangxi  | Han          | 27.7±4.4 | blood | convenient      | 564(female)              | RT-PCR | AA 409 | AC 144 | CC 11 | 166 |
| Ma LM  | 2015            | Heilongjiang | Han       | 28.1±5.5 | blood | convenient      | 455(female)              | RT-PCR | AA 336 | AC 110 | CC 9 | 128 |

(Continued)
### Table 2. Distribution of the MTHFR 677C>T polymorphism among populations in China.

| Author  | Publication Year | Location  | Ethnic Group | Age     | Sample Collection | Sample Size (Female/Male) | Method | Genotype |
|---------|------------------|-----------|--------------|---------|-------------------|---------------------------|--------|----------|
| Tang HY | 2014             | Shandong   | Han          | 27.7±3.8| Buccal cells       | 787 (female)              | RT-PCR | AA 607   |
| Tian Y  | 2014             | Jiangsu    | Han          | 27.0±4.8| Buccal cells       | 524 (female)              | RT-PCR | AC 361   |
| Lu GR   | 2014             | Shandong   | Han          | 28.5±5.0| Buccal cells       | 1352 (female)             | RT-PCR | CC 1027  |
| Jiao FY | 2014             | Shandong   | Han          | 28.2±4.2| Buccal cells       | 529 (female)              | RT-PCR |           |
| Gao X   | 2014             | Hebei      | Han          | 28.3±4.3| Buccal cells       | 860 (female)              | RT-PCR |           |
| Luo SQ  | 2014             | Jiangsu    | Miao         | 27.0±4.8| Buccal cells       | 518 (female)              | RT-PCR |           |
| Li XX   | 2015             | Jiangsu    | Han          | 26.7±3.6| Buccal cells       | 4008 (female)             | RT-PCR |           |
| Wang SY | 2015             | Hunan      | Han          | 26.7±4.6| Buccal cells       | 1701 (female)             | RT-PCR |           |
| Wu WQ   | 2015             | Jiangsu    | Han          | 26.4±4.5| Buccal cells       | 644 (female)              | RT-PCR |           |
| Mao WC  | 2015             | Guizhou    | Mixed        | 28.9±4.7| Buccal cells       | 1253 (female)             | RT-PCR |           |
| Cui HL  | 2015             | Henan      | Han          | 29.4±5.3| Buccal cells       | 443 (female)              | RT-PCR |           |
| Liu XL  | 2015             | Ningxia    | Han          | 28.5±4.3| Buccal cells       | 2620 (female)             | RT-PCR |           |
| Yu YH   | 2015             | Jilin      | Han          | 28.5±4.3| Buccal cells       | 2620 (female)             | RT-PCR |           |

### Table 3. Distribution of the MTRR A66G polymorphism among populations in China.

| Author  | Publication Year | Location  | Ethnic Group | Age     | Sample Collection | Sample Size (Female/Male) | Method | Genotype |
|---------|------------------|-----------|--------------|---------|-------------------|---------------------------|--------|----------|
| He XM   | 2010             | Mixed     | Han          | 19–46   | Blood populational-based | 1017 (female)             | RFLP   | AA 567   |
| Zhang QF| 2010             | Hainan    | Mixed        | 19–46   | Buccal cells       | 1008 (female)             | RT-PCR | AG 516   |
| Lao HH  | 2011             | Hainan    | Not given    | 19–44   | Buccal cells       | 11437 (female)            | RT-PCR | GG 5616  |
| Zhang Y | 2012             | Sichuan   | Han          | Not given| Buccal cells       | 2573 (female)             | RT-PCR |          |
| He YX   | 2012             | Henan     | Not given    | 19–44   | Buccal cells       | 1093 (female)             | RT-PCR |          |
| Yang Y  | 2012             | Jiangsu   | Han          | 27.0±4.4| Buccal cells       | 2885 (female)             | RT-PCR |          |
| Cong YY | 2012             | Shandong  | Han          | 29.4±7.7| Buccal cells       | 1041 (female)             | RT-PCR |          |
| Zhang YL| 2012             | Shandong  | Han          | 28.7±5.8| Buccal cells       | 825 (female)              | RT-PCR |          |
| Gao LJ  | 2012             | Guangdong | Han          | 27.6±4.0| Buccal cells       | 359 (female)              | RT-PCR |          |
| Yang BY | 2013             | Mixed     | Han          | 18–47   | Blood populational-based | 15357 (952/14405)         | RT-PCR |          |
| Xiu X   | 2013             | Shandong  | Han          | 19–40   | Buccal cells       | 2934 (female)             | RT-PCR |          |
| Wang WP | 2013             | Hubei     | Han          | 28.2±3.3| Buccal cells       | 2899 (female)             | RT-PCR |          |
| Gao H   | 2013             | Hubei     | Mixed        | 18–53   | Buccal cells       | 1902 (female)             | RT-PCR |          |
| Wan LJ  | 2013             | Hainan    | Han          | 27.2±5.3| Buccal cells       | 297 (female)              | RT-PCR |          |
| Yan ZM  | 2013             | Hainan    | Han          | 27.2±5.3| Buccal cells       | 1221 (female)             | RT-PCR |          |
| Lu XC   | 2013             | Guangxi   | Zhuang       | Mixed   | Buccal cells       | 300 (female)              | RT-PCR |          |
| Huang GX| 2013             | Hainan    | Mixed        | 28.5±5.3| Buccal cells       | 300 (female)              | RT-PCR |          |
| Luo XL  | 2014             | Hubei     | Han          | 27.3±5.2| Buccal cells       | 1077 (female)             | RT-PCR |          |

(Continued)
| Author     | Publicationyear | Location | Ethnicgroup | Age          | Sample | Sample collection | Sample size (male/female) | Method | Genotype | G allelic |
|------------|----------------|----------|-------------|--------------|--------|------------------|---------------------------|--------|----------|-----------|
| Wang FX    | 2014           | Shanxixi | Han         | 22–35        | buccal cells | convenient      | 1508(female)              | RT-PCR | 820      | 595       | 92        | 780       |
| Hao YY     | 2014           | Xinjiang | mixed       |              | buccal cells | convenient      | 210(female)               | RT-PCR | 96       | 91        | 23        | 137       |
| Yan Q      | 2014           | Shandong | Han         | 28.8±3.4     | buccal cells | convenient      | 2670(female)              | RT-PCR | 1459     | 1018      | 193       | 1404      |
| Xing JF    | 2014           | Henan    | Han         | 28.2±4.2     | buccal cells | convenient      | 425(female)               | RT-PCR | 241      | 162       | 19        | 200       |
| Jia XP     | 2015           | Sichuan  | Han         | 25.4±4.3     | buccal cells | convenient      | 4865(female)              | RT-PCR | 2748     | 1795      | 322       | 2439      |
| Huang QH   | 2015           | Jiangsu  | Han         | 26.5±4.3     | buccal cells | convenient      | 348(female)               | RT-PCR | 217      | 118       | 12        | 142       |
| Li JH      | 2015           | Hebei    | Han         | 27.3±4.9     | buccal cells | convenient      | 1267(female)              | RT-PCR | 705      | 496       | 66        | 628       |
| Xiang CG   | 2015           | Sichuan  | Han         | 26.0±4.8     | buccal cells | convenient      | 656(female)               | RT-PCR | 371      | 239       | 46        | 331       |
| Jiang W    | 2014           | Guangxi  | mixed       | 28.0±4.5     | buccal cells | convenient      | 948(female)               | RT-PCR | 501      | 376       | 71        | 518       |
| Chen XL    | 2014           | Guangxi  | Han         | 27.7±4.4     | buccal cells | convenient      | 564(female)               | RT-PCR | 324      | 209       | 31        | 271       |
| Ma LM      | 2015           | Heilongjiang | Hungarian   | 28.1±5.5     | buccal cells | convenient      | 455(female)               | RT-PCR | 245      | 184       | 26        | 236       |
| Tang HY    | 2014           | Shandong | Han         | 27.7±3.8     | buccal cells | convenient      | 787(female)               | RT-PCR | 444      | 288       | 55        | 398       |
| Tian Y     | 2014           | Jiangsu  | Han         | 27.0±4.8     | buccal cells | convenient      | 524(female)               | RT-PCR | 298      | 191       | 35        | 261       |
| Lu GR      | 2014           | Shandong | Han         | 28.5±5.0     | buccal cells | convenient      | 1352(female)              | RT-PCR | 779      | 498       | 75        | 648       |
| Jiao FY    | 2014           | Shandong | Han         | 28.2±4.2     | buccal cells | convenient      | 529(female)               | RT-PCR | 285      | 200       | 44        | 288       |
| Gao X      | 2014           | Hebei    | Han         | 28.3±4.3     | buccal cells | convenient      | 860(female)               | RT-PCR | 460      | 334       | 66        | 530       |
| Luo SQ     | 2015           | Guangxi  | Miao        | not given    | buccal cells | convenient      | 818(female)               | RT-PCR | 410      | 343       | 65        | 473       |
| Yu YH      | 2015           | Jilin    | Han         | 28.5±4.3     | buccal cells | convenient      | 2620(female)              | RT-PCR | 1479     | 977       | 164       | 1305      |
| Li XX      | 2015           | Jiangsu  | Han         | 26.7±3.6     | buccal cells | convenient      | 4008(female)              | RT-PCR | 2179     | 1543      | 286       | 1057      |
| Wang SY    | 2015           | Hunan    | Han         | 26.7±4.6     | buccal cells | convenient      | 1701(female)              | RT-PCR | 918      | 668       | 115       | 898       |
| Wu WQ      | 2015           | Jiangsu  | Han         | 26.4±5.5     | buccal cells | convenient      | 644(female)               | RT-PCR | 343      | 260       | 41        | 342       |
| Mao WC     | 2015           | Guizhou  | mixed       | not given    | buccal cells | convenient      | 1232(female)              | RT-PCR | 718      | 437       | 76        | 590       |
| Cui HL     | 2015           | Henan    | Han         | 28.9±4.7     | buccal cells | convenient      | 1253(female)              | RT-PCR | 704      | 481       | 68        | 617       |
| Liu XL     | 2014           | Ningxia | Han         | 29.4±5.3     | buccal cells | convenient      | 443(female)               | RT-PCR | 247      | 169       | 27        | 223       |
| Hu XW      | 2015           | Hubei    | NA          | 28.2±4.2     | buccal cells | convenient      | 3963(female)              | RT-PCR | 2247     | 1470      | 246       | 2962      |
| Polymorphisms | Genetic model | No. of studies | No. of provinces | No. of frequencies | Investigated number | Prevalence (95% CI) | Heterogeneity |
|---------------|---------------|----------------|------------------|-------------------|---------------------|---------------------|--------------|
| MTHFR C677T   | TT vs. total genotypes | 66             | 23               | 18302             | 92277               | 0.20 (0.18–0.23) | 100.0 0.000  |
|               | Allele contrast | 66             | 23               | 73823             | 184554              | 0.42 (0.38–0.45) | 100.0 0.000  |
| MTHFR A1298C  | CC vs. total genotypes | 51             | 18               | 4051              | 85616               | 0.05 (0.04–0.05) | 100.0 0.000  |
|               | Allele contrast | 51             | 18               | 33649             | 171232              | 0.20 (0.18–0.22) | 100.0 0.000  |
| MTTR A66G     | GG vs. total genotypes | 43             | 16               | 5957              | 84636               | 0.07 (0.06–0.07) | 100.0 0.000  |
|               | Allele contrast | 43             | 16               | 44508             | 169272              | 0.26 (0.25–0.28) | 100.0 0.000  |

Table 5. Summarized prevalence with 95% confidence intervals of genetic polymorphisms in the folate pathway with geographical distribution among Chinese populations.

| Polymorphisms | Latitude    | Genetic model | No. of studies | No. of provinces | No. of frequencies | Investigated number | Prevalence (95%CI) | Heterogeneity |
|---------------|-------------|---------------|----------------|------------------|-------------------|---------------------|---------------------|--------------|
| MTHFR C677T   | southern China | TT vs. total genotypes | 20             | 7                | 2131              | 27332               | 0.07 (0.05–0.08) | 100.0 0.000  |
|               | central China | TT vs. total genotypes | 19             | 6                | 7588              | 39205               | 0.19 (0.16–0.21) | 100.0 0.000  |
|               | northern China | TT vs. total genotypes | 27             | 10               | 8557              | 25569               | 0.28 (0.25–0.31) | 100.0 0.000  |
| MTHFR A1298C  | southern China | CC vs. total genotypes | 13             | 4                | 1705              | 26653               | 0.07 (0.05–0.09) | 100.0 0.000  |
|               | central China | CC vs. total genotypes | 19             | 7                | 1432              | 38936               | 0.04 (0.03–0.04) | 100.0 0.000  |
|               | northern China | CC vs. total genotypes | 19             | 7                | 692               | 19029               | 0.03 (0.02–0.03) | 100.0 0.000  |
| MTTR A66G     | southern China | GG vs. total genotypes | 4              | 3                | 402               | 4839                | 0.08 (0.06–0.10) | 100.0 0.000  |
|               | central China | GG vs. total genotypes | 19             | 6                | 2710              | 41728               | 0.06 (0.06–0.07) | 100.0 0.000  |
|               | northern China | GG vs. total genotypes | 20             | 7                | 1192              | 19759               | 0.06 (0.05–0.06) | 100.0 0.000  |
45%) in central, and 28% (25%-31%) and 53% (51%-55%) in northern China, respectively. There were significant geographical gradients in the variations in the frequencies of the 677T allele and 677TT genotype (both \( P \) values ≤0.001).

The frequency of the MTHFR A1298C polymorphism exhibited the reverse trend; i.e., this frequency decreasing from southern to central to northern China. The pooled geographical gradient frequencies of the 1298C allele and 1298CC genotype were found to be 28% (24%-31%) and 7% (5%-9%) in southern, 18% (17%-19%) and 4% (3%-4%) in central, and 17% (16%-19%) and 3% (2%-3%) in northern China, respectively (Table 5). There were significant geographical gradients in the frequencies of the 1298C allele and 1298CC genotype (both \( P \) values ≤0.001).

The mean frequencies of the MTRR 66G allele and 66GG genotype decreased from 29% (28%-30%) and 8% (6%-10%) in southern China, to 25% (23%-27%) and 6% (6%-7%) in central China, and 24% (23%-25%) and 6% (5%-6%) in northern China (Table 5) in a pattern similar to that observed in the gradients of the MTHFR 1298C allele and 1298CC genotype frequencies (both \( P \) values ≤0.001).

The frequencies of the MTHFR C677T, A1298C, and MTRR A66G polymorphisms by ethnicity

The allele and genotype distributions of MTHFR and MTRR by ethnicity are presented in Table 6. The distributions of the MTHFR 677T allele and the 677TT genotype exhibited ethnic variations (with both \( P \) values ≤0.001). The 677T allele frequencies in the minority groups (e.g., Miao, Zhuang, She, Shui, etc.) and Chinese Han were 28% (25%-31%) and 45% (41%-49%), respectively. The 677TT genotype frequencies in the minority groups and Chinese Han were 5% (4%-6%) and 22% (20%-25%), respectively.

In contrast to C677T, the distribution of the A1298C polymorphism by ethnicity demonstrated the reverse trend: the 1298C allele was much more common among the minority groups [26%, (23%-30%)] than the Chinese Han [19% (17%-20%); \( P \) value ≤0.001]. The 1298CC genotype exhibited similar variability with frequencies of 7% (5%-9%) in the minority groups and 4% (3%-5%) in the Chinese Han (\( P \) value ≤0.001).

The frequencies of the MTRR 66G allele and 66GG genotype varied by ethnic group and geographical location. The frequency of the 66G allele was slightly higher among the minority groups [35% (35%-36%)] compared with 25% (24%-26%) among the Chinese Han group (\( P \) value ≤0.001). The frequencies of the 66GG genotype were 10% (8%-12%) in the minority groups and 6% (5%-7%) in the Chinese Han group, which were similar to those of the MTHFR A1298C polymorphism (\( P \) value ≤0.001).

The frequencies of MTHFR C677T, A1298C and MTRR A66G polymorphisms by sex

Table 7 provides the pooled frequencies of the variant alleles and genotypes of MTHFR C677T and A1298C and MTRR A66G according to sex. A total of 88255 samples with reported C677T polymorphisms were obtained. Based on all these samples, we did not find any difference between the males [19% (12%-25%)] and females [21% (19%-24%)] in terms of 677TT genotype frequency.

Only 41 studies reported the frequency of the MTHFR A1298C polymorphism and included 82352 females of reproductive age. The 1298C allele and 1298CC genotype frequencies in females were 19% (18%-21%) and 4% (4%-5%), respectively. Among the 43 articles that reported on the MTRR A66G polymorphism, 42 studies included 84416 females. The 66G allele and 66GG genotype frequencies in females were 26% (25%-27%) and 7% (6%-7%), respectively.
### Table 6. Summarized prevalence with 95% confidence intervals of genetic polymorphisms in the folate pathway with ethnicity distribution among Chinese populations.

| Polymorphisms | Ethnicity | Genetic model | No. of studies | No. of ethnic groups | No. of provinces | No. of frequencies | Investigated number | Prevalence(95% CI) | Heterogeneity |
|---------------|----------|---------------|----------------|----------------------|-----------------|-------------------|---------------------|------------------|---------------|
|               |          |               |                |                      |                 |                   |                     |                  | I²(%) | P            |
| MTHFR C677T   | Minority | TT vs. total genotypes | 17          | 19                  | 11              | 381               | 7559                | 0.05(0.04–0.06)    | 100.0 | 0.000     |
|               |          | Allele contrast  | 17          | 19                  | 11              | 3390              | 15118               | 0.28(0.25–0.31)    | 100.0 | 0.000     |
| Han           |          | TT vs. total genotypes | 55          | 1                   | 22              | 16973             | 78852               | 0.22(0.20–0.25)    | 100.0 | 0.000     |
|               |          | Allele contrast  | 55          | 1                   | 22              | 66065             | 157704              | 0.45(0.41–0.49)    | 100.0 | 0.000     |
| MTHFR A1298C  | Minority | CC vs. total genotypes | 10          | 8                   | 4               | 368               | 4669                | 0.07(0.05–0.09)    | 100.0 | 0.000     |
|               |          | Allele contrast  | 10          | 8                   | 4               | 2494              | 9338                | 0.26(0.23–0.30)    | 100.0 | 0.000     |
| Han           |          | CC vs. total genotypes | 44          | 1                   | 17              | 3228              | 74454               | 0.04(0.03–0.05)    | 100.0 | 0.000     |
|               |          | Allele contrast  | 44          | 1                   | 17              | 28205             | 148908              | 0.19(0.17–0.20)    | 100.0 | 0.000     |
| MTRR A66G     | Minority | GG vs. total genotypes | 8           | 5                   | 4               | 436               | 4792                | 0.10(0.08–0.12)    | 100.0 | 0.000     |
|               |          | Allele contrast  | 8           | 5                   | 4               | 2918              | 9584                | 0.35(0.35–0.36)    | 100.0 | 0.000     |
| Han           |          | GG vs. total genotypes | 39          | 1                   | 15              | 5210              | 75357               | 0.06(0.05–0.07)    | 100.0 | 0.000     |
|               |          | Allele contrast  | 39          | 1                   | 15              | 38367             | 150714              | 0.25(0.24–0.26)    | 100.0 | 0.000     |

### Table 7. Summarized prevalence with 95% confidence intervals of genetic polymorphisms in the folate pathway with sex distribution among Chinese populations.

| Polymorphisms | Gender | Genetic model | No. of studies | No. of provinces | No. of frequencies | Investigated number | Prevalence(95% CI) | Heterogeneity |
|---------------|--------|---------------|----------------|-----------------|-------------------|---------------------|------------------|---------------|
|               |        |               |                |                 |                   |                     |                  | I²(%) | P            |
| MTHFR C677T   | Male   | TT vs. total genotypes | 11          | 6                | 462               | 2507                | 0.19(0.12–0.25)   | 100.0 | 0.000     |
|               |        | Allele contrast  | 11          | 6                | 2380              | 5014                | 0.49(0.41–0.58)   | 100.0 | 0.000     |
|               | Female | TT vs. total genotypes | 53          | 17               | 17311             | 85748               | 0.21(0.19–0.24)   | 100.0 | 0.000     |
|               |        | Allele contrast  | 53          | 17               | 69098             | 171496              | 0.44(0.40–0.47)   | 100.0 | 0.000     |
| MTHFR A1298C  | Female | CC vs. total genotypes | 41          | 17               | 3733              | 82532               | 0.04(0.04–0.05)   | 100.0 | 0.000     |
|               |        | Allele contrast  | 41          | 17               | 31883             | 165064              | 0.19(0.18–0.21)   | 100.0 | 0.000     |
| MTRR A66G     | Female | GG vs. total genotypes | 42          | 15               | 5907              | 84416               | 0.07(0.06–0.07)   | 100.0 | 0.000     |
|               |        | Allele contrast  | 42          | 15               | 44351             | 168832              | 0.26(0.25–0.27)   | 100.0 | 0.000     |

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Publication bias

Tables 4–7 presents information related to heterogeneity and publication bias. We noted significant heterogeneity within the studies and the subgroups (all P values were ≤0.001, I² = 100.0).

Discussion

Methylenetetrahydrofolate reductase (MTHFR) (C677T and A1298C) and methionine synthase reductase (MTRR) mutations (A66G) cause mild hyperhomocysteinemia and low folate level and are associated with several disorders. The geographical and ethnic distributions of these alleles and the associated genotypes are important to study worldwide.

The frequencies of the MTHFR C677T and A1298C and MTRR A66G polymorphism in 68 epidemiological studies covering 23 provinces in Mainland China were pooled and investigated in the present study. Currently, there is a lack of national data regarding the prevalences of gene polymorphisms in the folate metabolism pathway in healthy general populations in China. We documented distinctive geographical and ethnic variations in the frequencies of the C677T and A1298C polymorphisms of the MTHFR gene and the A66G polymorphisms of the MTRR gene among nation-wide samples in China.

Worldwide data have revealed that significant heterogeneities in the frequencies of the T allele and TT homozygosity exist in every population and even with racial groups. One investigations conducted in Texas reported that the frequency of the 677T was lowest among African-Americans (11.9%), followed by in Caucasians (32.7%) and Ashkenazi Jews (47.7%), and the highest frequency exists among the Hispanic population (47.9%) [86]. In the Chinese Han, the frequencies of the 677T allele have been found to be lowest in Hainan (24.0%) followed by Hubei (40.3%) and Jiangsu (43.5%), and the highest frequency has been observed in Shandong (63.1%) [51].

Population genetic comparisons provide an appropriate method for picturing geographical and ethnic variations and can suggest that environmental factors may exert selective pressures on genetic mutations. A north-to-south increase in the frequency of the 677T allele has been observed in Europe [87]. North-to-south increases in dietary folate intake have also been encountered in European populations [88]. Thus adequate folic acid intakes have presumed enabled increase in the MTHFR 677T frequency in these populations [89]. Economic and dietary habits might have played important roles in the spread of the 677T allele worldwide. For example, the frequency of the 677T allele is high in the USA with an average frequency of 36.2% in Texas [86]. Another study conducted in India observed the highest frequency of the 677T allele among the Sindhi population (23.8%). In contrast, the 677T allele is absent in the Kom, Thadou and Munda populations, and its average frequency is 10.1% across all 23 populations in India [10]. The low frequencies of the 677T allele among the tribal groups (i.e., the Kom, Thadou and Munda populations) may have been influenced by folate deficiencies because the majority of the population in India has a vegetarian diets that is low in vitamin B₁₂ [10]. The populations of America carried higher frequencies of the 677T allele, which may be related to abundant nutritional statuses and particularly with folic acid and vitamin B₁₂ supplementation, which are associated with low levels of homocysteinemia. Across all 23 of the studied provinces, we observed increases in the 677T allele and 677TT genotype frequencies in the southern-central-northern direction across Mainland China. Because high 677T allele and 677TT genotype frequencies were observed in the northern populations, we assumed that the folic acid intakes are greater in the northern populations than in the southern populations; however, the opposite pattern has been observed in nutritional studies. One such nutritional investigation revealed that the geometric mean of the blood folate concentration is lower in the northern populations than the southern populations [90].
Worldwide epidemiological data have revealed that the frequency of A1298C homozygosity varies from continent to continent. The frequencies of the 1298C allele range from 18% to 70% in East Asia, 17% to 44% in Asia, 24% to 40% in Europe, 0% to 15% in South America and 14.7% in North America [91]. The present data revealed variation in the frequency of the 1298C allele within China. In contrast to the distribution of 677T, the frequency of the 1298C allele was found to be the lowest in northern China [18% (17%-19%)], intermediate in central China [18% (17%-19%)], and highest in southern China [28% (24%-31%)]. The mean frequency of the 1298C allele was 20% (18%-22%).

Based on all 8 of the investigated minority ethnic populations (e.g., the She, Xibo, and Uygur), the minority ethnic populations seemed to carry greater 1298C allele frequencies than the Chinese Han population. Notably, the frequency of the 1298C allele has been reported to vary between different ethnic populations worldwide, and the lowest frequency has been found in Indians (10%) [92] followed by the Chinese (18.4%) [51] and Tamils (35%) [93], and the highest frequency has been observed in the Lebanese [94].

Although A1298C homozygotes do not exhibit elevated blood homocysteinemia levels, many investigations have revealed that compound heterozygotes for C677T/ A1298C may be at risk for hyperhomocysteinemia and low folate levels, which can contribute to many disorders, such as neural tube defects [6] and abortions [95].

Because lifestyle and environmental factors, such as folate supplementation, vary across different ethnic populations and may influence the frequencies of the C677T and A1298C alleles, these factors cannot be ruled out when considering the influences of environmental-genetic interactions on the distributions of MTHFR gene polymorphisms.

Our pooled data revealed that the frequencies of the 66G allele and 66GG genotype exhibited variations across geographical gradients and ethnic populations. Globally, the distributions of the MTRR 66G allele and 66GG genotype frequencies also exhibit geographical and ethnic variations. For example, the frequencies of the 66G allele have been reported to be 58% in the Yadav, 62% in the Scheduled Castes, and 71% in the rural Sunni Muslim population in Uttar Pradesh in India [96,97]. Our study observed a 66GG genotype frequency of 7% across Mainland China, which is much lower than those in Brazil (23%), Australia (10%), and Ireland (17.5%) [98–100]. MTRR is involved in the homocysteine and folate metabolic pathway via its activation of methionine synthase via reductive methylation and is consequently a critical determinant of homocysteinemia levels [101]. Therefore, the MTRR A66G mutation may indirectly contribute to many medical disorders, such as neural tube defects and congenital heart disease [102], due to its key role in the folate metabolism pathway. However, due to limited sample sizes and the lower frequency of studies of the A66G polymorphisms in MTRR, no solid evidence has been found to relate the MTRR A66G variant with the risks of diseases. Long-term data and larger sample sizes are necessary to determine the real connections between the distribution of the A66G variant and the risks of diseases.

Conclusions
In conclusion, our meta-analysis revealed significant geographical variations in the frequencies of the MTHFR C677T and A1298C and MTRR A66G polymorphisms in the folate metabolism pathway between different ethnic populations in China. Our findings provide an overall picture of these three genetic polymorphisms in the folate metabolism pathway among the general populations in Mainland China, and these evidence-based genomic data should be integrated into medical and public health practices.
Supporting Information

S1 File. The average frequencies of the 677TT genotype in the healthy Chinese population.

S2 File. The average frequencies of the 677T allele in the healthy Chinese population.

S3 File. The average frequencies of the 1298CC genotype in the healthy Chinese population.

S4 File. The average frequencies of the 1298C allele in the healthy Chinese population.

S5 File. The average frequencies of the 66GG genotype in the healthy Chinese population.

S6 File. The average frequencies of the 66G allele in the healthy Chinese population.

Author Contributions

Conceived and designed the experiments: DYZ. Performed the experiments: XMW JJF. Analyzed the data: QXL JJF. Contributed reagents/materials/analysis tools: XMW JJF QXL DYZ. Wrote the paper: XMW JJF. Guided the writing: QXL. Revised the manuscript: DYZ.

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