The association between 5, 10 – methylenetetrahydrofolate reductase and the risk of unexplained recurrent pregnancy loss in China
A Meta-analysis

Genzhu Wang, PhD, Zhaohui Lin, BS, Xiaoying Wang, PhD, Qiang Sun, MS, Zhikun Xun, BS, Baiqian Xing, MS, Zhongdong Li, MD, PhD

Abstract
Background: To analyze the correlation between gene polymorphisms of 5,10- methylenetetrahydrofolate reductase (MTHFR) and risk of unexplained recurrent pregnancy loss (URPL) in Chinese women.

Methods: Eligible studies were searched in Pubmed, Embase, Web of Science, Wanfang, and China National Knowledge Infrastructure (CNKI) databases. Established inclusion criteria were used to screening articles, subsequently evaluate the quality of the included studies, Stata 16.0 PM and RevMan 5.3 software were conducted for meta-analysis. The pooled odds ratio (OR) with 95% confidence interval (CI) was determined to assess the relationship between MTHFR and risk of URPL in Chinese women.

Results: For MTHFR C677T, fifty studies were included, involving 6677 URPL cases and 8111 controls. The overall results showed that MTHFR C677T was significantly correlated with URPL risk, especially in the homozygous model (TT vs CC; OR 3.06; 95% CI 2.56–3.66). For MTHFR A1298C, twenty-first studies were included, involving 3439 URPL cases and 3155 controls. The results showed that MTHFR A1298C was also significantly correlated with URPL risk in recessive (CC vs AC + AA; OR 1.55; 95% CI 1.25–1.93) and homozygous (CC vs AA; OR 1.53; 95% CI 1.22–1.91) models. In addition, sub-group results showed that no significant difference between north and south China populations in the MTHFR gene polymorphisms and URPL risk. Of note, the patients carrying MTHFR C677T and MTHFR A1298C joint mutants had no synergistic effect (OR 2.71; 95% CI 0.84–8.70) on the occurrence of URPL compared with the wild-type homozygous genotype (MTHFR 677CC/ MTHFR 1298AA).

Conclusion: Studies included in this meta-analysis suggested that MTHFR 677T allele and 677TT genotype and MTHFR 1298CC genotype were both associated with URPL; testing MTHFR C677T gene polymorphism was a more appropriate target compared with other mutations in the prediction of URPL.

Abbreviations: CI = confidence interval, CNKI = China National Knowledge Infrastructure, FEM = fixed effects model, HWE = Hardy–Weinberg equilibrium, MTHFR = 5,10-methylenetetrahydrofolate reductase, 5,10-MTHF = 5,10-methylenetetrahydrofolate, 5-MTHF = 5-methyltetrahydrofolate, OR = odds ratio, PRISMA = the Preferred Reporting Items for Systematic Reviews and Meta-Analyses, RPL = recurrent pregnancy loss, NOS = Newcastle-Ottawa Scale, REM = random effects model, URPL = unexplained recurrent pregnancy loss.

Keywords: 5, 10 – methylenetetrahydrofolate reductase, unexplained recurrent pregnancy loss, meta-analysis
1. Introduction

Recurrent pregnancy loss (RPL) was defined as two or more failed clinical pregnancies by American Society for Reproductive Medicine.[1] A total of 1–5% of women in reproductive age will experience RPL.[2,3] Although abnormalities of the genital tract and the uterine structure, autoimmune diseases, genetic disorders, and inherited thrombophilia had been identified as risk factors for RPL, nearly 50% of RPL cases were still classified as URPL.[4] MTHFR C677T and MTHFR A1298C of which were the two most-investigated single nucleotide polymorphisms.[8–11]

Numerous case-control studies and meta-analyses reported that MTHFR C677T was significantly associated with URPL.[10–12] The frequencies of the MTHFR 677TT allele and 677TT genotype increased along the south-north direction, and showed significantly geographical variations among Chinese population.[13–15] However, to our best knowledge, no meta-analysis compared the relationship between MTHFR C677T geographical distribution and the risk of URPL by meta-analysis.

The correlations between MTHFR A1298C and the risk of URPL were also reported by lots of meta-analyses. However, these results were controversial.[8–11] Three meta-analyses indicated MTHFR A1298C polymorphisms were significantly associated with URPL,[9,11,16] whereas others showed no significant correlation between them.[8,10] Considered that a series of novel case-control studies about Chinese women’s URPL had been published, an updated meta-analysis needed to further validate the association between MTHFR A1298C and the risk of URPL.

The frequencies of MTHFR 1298C allele and 1298CC genotype decreased along the southern-northern direction, and also showed significantly geographical variations.[13,14] Hence, it is also important to clarify the correlation between MTHFR A1298C geographical distributions and the risk of URPL by meta-analysis.

The MTHFR 677T allele and the 677TT genotype reduced the activity of MTHFR, and the MTHFR 1298C allele and the 1298CC genotype increased folate level in the blood.[9] To our best knowledge, no meta-analysis investigates the association between MTHFR C677T and MTHFR A1298C joint mutation and the risk of URPL.

Therefore, we performed a comprehensive and updated meta-analysis to drive a precise estimation of association between MTHFR C677T or MTHFR A1298C gene polymorphisms and the risk of URPL in Chinese women, to unravel the effects of geographical variations of MTHFR C677T or MTHFR A1298C gene polymorphisms on the risk of URPL, and to analyze the association between MTHFR C677T and MTHFR A1298C joint mutation and the risk of URPL. The findings of this meta-analysis may help predict the risk of URPL in Chinese women from the angle of MTHFR C677T or/and MTHFR A1298C gene polymorphisms.

2. Materials and methods

The meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[17] This review has been registered on the PROSPERO website as No. CRD42020173815 (To enable PROSPERO to focus on COVID-19 registrations during the 2020 pandemic, this registration record was automatically published exactly as submitted. The PROSPERO team has not checked eligibility).

2.1. Search strategy

A systematic literature search for studies published up to March 25, 2020, was performed. Relevant studies were identified by searching Pubmed, Embase, Web of Science, Wanfang, and CNKI databases with the following key words: ‘Chinese’ or ‘China’, ‘methylenetetrahydrofolate reductase’ or ‘MTHFR’, ‘recurrent miscarriage’ or ‘recurrent abortion’ or ‘spontaneous abortion’ or ‘recurrent pregnancy loss’ or ‘recurrent fetal loss’. Only studies that had been published in English or Chinese were included.

2.2. Inclusion and exclusion criteria

Studies were selected according to the following criteria:

1. case-control studies;
2. all the participants were Chinese women. URPL was defined as at least two miscarriages, and the controls were women with at least one live birth;
3. MTHFR C677T and/or MTHFR A1298C gene polymorphisms were detected and sufficient data regarding genotype distributions were provided; and
4. articles were published in English or Chinese.

For duplicates, only the studies with more complete data were included. Case reports, comments, review papers were excluded. The full-text screening according to the inclusion and exclusion procedures was summarized in Figure 1.

2.3. Data extraction

The following information from each eligible publication was recorded: first name of the first author, year of publication, province, sample size, number of cases and controls with genotypes, the genotype frequencies of control group were evaluated for Hardy–Weinberg equilibrium (HWE), and the quality of included studies was estimated by the 9-star Newcastle-Ottawa Scale (NOS).[18] The stars of 0–3, 4–6, and 7–9 were considered of low, moderate, and high quality, respectively.

2.4. Data analysis

All statistical analyses were performed using the STATA 16.0PM and RevMan 5.3 software. The two-tailed Student t test was employed to analyze the geographical distribution data, $P < .05$ was considered statistically significant. The strength of the association between the MTHFR gene polymorphisms (C677T and A1298C) and URPL risk in the Chinese women was assessed by the odds ratios (ORs) with 95% confidence intervals (CIs). For MTHFR C677T, the pooled ORs were calculated for the dominant model (TT+CT vs CC), recessive model (TT vs CT+CC), heterozygous model (CT vs CC), homozygous model (TT vs CC), and an allele model (T vs C); for MTHFR A1298C, the pooled ORs were also calculated for the dominant model (CC+AC vs AA), recessive model (CC vs AC+AA), heterozygous
model (AC vs AA), homozygous model (CC vs AA), and an allele model (C vs A). χ² and Higgins I² statistics were performed to assess heterogeneity between studies. If $I^2 > 50\%$ and $P < .01$, the pooled ORs were analyzed using the random effects model (REM), or else, the fixed effects model (FEM) was used. Publication bias was estimated by Egger’s linear regression test. If $P < .05$, a significant publication bias was considered.

3. Results

3.1. Main characteristics and quality assessment of the included studies

Overall, fifty-three articles were included in the final meta-analysis. For MTHFR C677T, fifty studies with 6677 URPL cases and 8111 controls were included. The stars of NOS ranged from 5-9, and only two studies were classed as moderate quality. The main characteristics of all included studies were shown in Table 1. Twenty-one articles demonstrated the association of MTHFR A1298C polymorphism with risk of URPL involving 3439 URPL cases and 3155 controls. All studies were classed as high quality according to the NOS score. The main characteristics were shown in Table 2. Five studies investigated the relationship between the MTHFR joint mutations and the risk of URPL, all studies were classed as high quality, and the main characteristics were listed in Table 3.

3.2. Geographical distributions of MTHFR gene polymorphisms

The prevalence of the two gene polymorphisms varied significantly among different populations and showed apparent geographical gradients. For MTHFR C677T, the MTHFR 677TT genotype frequency was significantly higher in URPL group compared with control group ($P < .001$), and the frequency increased along the south-north direction. For MTHFR A1298C, the MTHFR 1298CC genotype frequency was slightly higher in URPL group compared with control (not statistically significant), and the frequency decreased along the south-north direction (Table 4).
3.3. MTHFR C677T and/or MTHFR A1298C gene polymorphisms and URPL risk

The ORs and 95% CIs of the association between MTHFR gene polymorphisms and URPL risk were considered under different models (Figure S1 and S2 http://links.lww.com/MD/GB, http://links.lww.com/MD/G9, http://links.lww.com/MD/G10, http://links.lww.com/MD/G11, http://links.lww.com/MD/G12, http://links.lww.com/MD/G13). Main meta-analyzed results of the association of MTHFR C677T and URPL risk were summarized in Table 5. The overall pooled results indicated significant
Table 2: Characteristics of all selected studies included on MTHFR A1298C.

| Author         | Province | Publication year | Cases No. | Control No. | HWE | P values | NOS score |
|----------------|----------|-----------------|-----------|-------------|-----|----------|-----------|
|               |          |                 |           |             |     |          |           |
| Li et al, [69] | Shandong | 2003            | ≥2        | 57          | 33  | 21 3     | Y .93     | 8         |
| Wang et al, [23] | Shanghai | 2006            | ≥2        | 148         | 103 | 35 10    | Y .83     | 7         |
| Ren et al, [24] | Shanghai | 2007            | ≥2        | 71          | 49  | 20 2     | Y .54     | 7         |
| Chen et al, [25] | Hainan    | 2013            | ≥2        | 59          | 24  | 29 6     | Y .09     | 8         |
| Hu et al, [26] | Guangdong | 2014            | ≥2        | 52          | 33  | 12 7     | Y .25     | 8         |
| Cao et al, [27] | Shanghai  | 2014            | ≥2        | 166         | 132 | 31 3     | Y .25     | 8         |
| Luo et al, [28] | Zhejiang  | 2015            | ≥2        | 125         | 82  | 40 3     | Y .07     | 9         |
| Zhu et al, [29] | Henan     | 2015            | ≥2        | 118         | 48  | 58 12    | Y .02     | 8         |
| Gao et al, [30] | Henan     | 2015            | ≥2        | 378         | 180 | 118 80   | Y .08     | 7         |
| Li et al, [31] | Jiangsu   | 2015            | ≥2        | 60          | 31  | 21 8     | Y .12     | 7         |
| Wang et al, [32] | Jiangsu  | 2016            | ≥2        | 190         | 77  | 93 19    | Y .01     | 9         |
| Xie et al, [33] | Tianjin   | 2016            | ≥2        | 244         | 165 | 74 5     | Y .25     | 8         |
| Hu et al, [34] | Guangdong | 2017            | ≥2        | 83          | 52  | 28 3     | Y .60     | 9         |
| Zhang et al, [35] | Shanghai | 2017            | ≥2        | 140         | 100 | 36 4     | Y .21     | 9         |
| Zhu et al, [36] | Zhejiang  | 2017            | ≥2        | 50          | 30  | 19 1     | Y .88     | 7         |
| Lin et al, [37] | Guangxi   | 2017            | ≥2        | 370         | 243 | 114 13   | Y .23     | 8         |
| Xu et al, [38] | Henan     | 2017            | ≥2        | 403         | 231 | 144 28   | Y .12     | 9         |
| Xu et al, [39] | Zhejiang  | 2017            | ≥2        | 218         | 155 | 58 5     | Y .05     | 9         |
| Liu et al, [40] | Jiangsu   | 2018            | ≥2        | 108         | 61  | 37 10    | Y .21     | 8         |
| Xu et al, [41] | Henan     | 2018            | ≥2        | 170         | 119 | 49 2     | Y .26     | 8         |
| Lin et al, [42] | Guangxi   | 2019            | ≥2        | 230         | 156 | 67 7     | Y .05     | 9         |
| Xu et al, [43] | Zhejiang  | 2019            | ≥2        | 410         | 44  | 28 18    | Y .72     | 9         |
| Liu et al, [44] | Henan     | 2020            | ≥2        | 188         | 57  | 96      | Y .72     | 9         |

MTHFR = 5,10-methylenetetrahydrofolate reductase, HWE = Hardy–Weinberg equilibrium. * Bold for north China, regular for south China. † Y: genotype frequencies of control group were meet HWE criteria; N: genotype frequencies of control group were not meet HWE criteria. ‡ P value for HWE in control group. x Scores estimated by the 9-star Newcastle–Ottawa Scale.

Table 3: Characteristics of all selected studies included on MTHFR joint mutations.

| Author         | Province | Publication year | Case No. | Control No. | HWE | P values | NOS score |
|----------------|----------|-----------------|----------|-------------|-----|----------|-----------|
|                |          |                 |          |             |     |          |           |
| Wang et al, [23] | Shanghai | 2006            | ≥2        | 23          | 27  | 7        | Y .61     | 8         |
| Zhang et al, [40] | Zhejiang | 2017            | ≥2        | 12          | 6   | 2        | Y .18     | 7         |
| Zhu et al, [37] | Guangxi   | 2017            | ≥2        | 48          | 88  | 23       | Y .32     | 9         |
| Xu et al, [38] | Henan     | 2019            | ≥2        | 41          | 4   | 28       | Y .72     | 9         |
| Xu et al, [41] | Henan     | 2020            | ≥2        | 49          | 4   | 28       | Y .72     | 9         |

MTHFR = 5,10-methylenetetrahydrofolate reductase, HWE = Hardy–Weinberg equilibrium, No. = number. * Y: genotype frequencies of control group were meet HWE criteria; N: genotype frequencies of control group were not meet HWE criteria. † P value for HWE in control group. ‡ Scores estimated by the 9-star Newcastle–Ottawa Scale.

Table 4: Distribution frequencies under different MTHFR gene polymorphisms.

| Groups | No. | Genotype frequencies [n (%)] |
|--------|-----|------------------------------|
|        |     | Wild type                    | Heterozygous type | Homozygous type |
|        | Cases |                            |                  |                |
| C677T  | Total | 6677                         | 1919 (28.84)     | 2957 (44.48)   |
|        | Control | 8111                        | 3318 (41.03)     | 3592 (44.26)   |
|        | North | 3725                         | 874 (23.46)      | 1673 (44.91)   |
|        | South | 2952                         | 1045 (35.40)     | 1284 (43.50)   |
|        | A1298C Total | 3439                        | 2206 (61.18)     | 1139 (32.10)   |
|        | North | 1686                         | 1029 (61.03)     | 530 (31.44)    |
|        | South | 1753                         | 1075 (61.32)     | 574 (32.74)    |

* P < .001 vs control, MTHFR = 5,10-methylenetetrahydrofolate reductase, No. = number.
associations between all the MTHFR C677T gene polymorphisms and the risk of URPL, especially homozygous model (TT vs CC; OR 3.06; 95% CI 2.56–3.66). When excluding studies that deviated from HWE, significant associations were also found in all these models (Table 5). The overall results of MTHFR A1298C polymorphism also showed significant association in the recessive (CC vs AC+AA; OR 1.55; 95% CI 1.25–1.93) and homozygous (CC vs AA; OR 1.53; 95% CI 1.22–1.91) models. When excluding studies that deviated from HWE, significant associations were still found in these two models (Table 6).

Through linkage analysis of the MTHFR C677T and A1298C loci, our results found that the patients carrying these two MTHFR mutants had no synergistic effect (OR 2.71; 95% CI 0.84–8.70) on the occurrence of URPL compared with the

### Table 5

| Contrast Group | No. | I2(%) | Model | Pooled OR(95% CI) Egger (P value) | P value |
|----------------|-----|-------|-------|-----------------------------------|---------|
| TT + CT vs CC  | 50  | 51.9  | REM   | 1.91 (1.70–2.15)                  | .61     |
| HWE: yes       | 38  | 49.7  | FEM   | 2.01 (1.84–2.19)                  | .75     |
| Distribution: North | 25  | 59.6  | REM   | 1.99 (1.65–2.39)                  | .57     |
| Distribution: South | 25  | 43.0  | REM   | 1.86 (1.67–2.07)                  |         |
| TT vs CT+CC    | 50  | 57.0  | REM   | 2.24 (1.93–2.60)                  | .03     |
| HWE: yes       | 38  | 58.5  | REM   | 2.16 (1.83–2.56)                  | .11     |
| Distribution: North | 25  | 69.1  | REM   | 2.16 (1.74–2.68)                  | .47     |
| Distribution: South | 25  | 31.2  | FEM   | 2.24 (1.92–2.61)                  |         |
| CT vs CC       | 50  | 50.7  | REM   | 1.59 (1.40–1.80)                  | .84     |
| HWE: yes       | 38  | 25.5  | FEM   | 1.70 (1.55–1.87)                  | .44     |
| Distribution: North | 25  | 53.9  | REM   | 1.63 (1.36–1.96)                  | .98     |
| Distribution: South | 25  | 49.4  | FEM   | 1.60 (1.42–1.80)                  |         |
| TT vs CC       | 50  | 57.8  | REM   | 3.06 (2.56–3.66)                  | .06     |
| HWE: yes       | 38  | 63.9  | REM   | 2.95 (2.38–3.67)                  | .28     |
| Distribution: North | 25  | 70.3  | REM   | 3.10 (2.33–4.11)                  | .91     |
| Distribution: South | 25  | 31.9  | FEM   | 2.96 (2.48–3.49)                  |         |
| T vs C         | 50  | 61.3  | REM   | 1.74 (1.60–1.90)                  | .70     |
| HWE: yes       | 38  | 69.2  | REM   | 1.75 (1.57–1.93)                  | .99     |
| Distribution: North | 25  | 69.8  | REM   | 1.74 (1.53–1.97)                  | .92     |
| Distribution: South | 25  | 49.3  | FEM   | 1.73 (1.60–1.87)                  |         |

OR = odds ratio, CI = confidence interval, MTHFR = 5,10-methylentetrahydrofolate reductase, URPL = unexplained recurrent pregnancy loss, No. = number, HWE = Hardy–Weinberg equilibrium, NOS = Newcastle–Ottawa Scale.

* REM: random effects model; FEM: fixed effects model.
† P value for north vs south.

### Table 6

| Contrast Group | No. | I2(%) | Model | Pooled OR(95% CI) Egger (P value) | P value |
|----------------|-----|-------|-------|-----------------------------------|---------|
| CC+AC vs AA    | 21  | 54.5  | REM   | 1.07 (0.90–1.26)                  | .06     |
| HWE: yes       | 17  | 43.1  | FEM   | 0.99 (0.88–1.12)                  | .64     |
| Distribution: North | 8   | 58.2  | REM   | 1.24 (0.95–1.56)                  | .10     |
| Distribution: South | 13  | 50.9  | REM   | 0.97 (0.78–1.24)                  |         |
| CC vs AA+AC    | 21  | 0.0   | FEM   | 1.55 (1.25–1.93)                  | .04     |
| HWE: yes       | 17  | 1.6   | FEM   | 1.54 (1.21–1.97)                  | .08     |
| Distribution: North | 8   | 9.6   | FEM   | 1.63 (1.21–2.19)                  | .64     |
| Distribution: South | 13  | 0.0   | FEM   | 1.47 (1.06–1.93)                  |         |
| AC vs AA       | 21  | 56.5  | REM   | 1.02 (0.85–1.21)                  | .75     |
| HWE: yes       | 17  | 39.3  | FEM   | 0.92 (0.81–1.05)                  | .97     |
| Distribution: North | 8   | 67.9  | REM   | 1.17 (0.87–1.58)                  | .15     |
| Distribution: South | 13  | 45.6  | FEM   | 0.95 (0.82–1.11)                  |         |
| CC vs AA       | 21  | 0.0   | FEM   | 1.53 (1.22–1.91)                  | .06     |
| HWE: yes       | 17  | 0.0   | FEM   | 1.49 (1.16–1.91)                  | .10     |
| Distribution: North | 8   | 0.0   | FEM   | 1.57 (1.15–2.13)                  | .82     |
| Distribution: South | 13  | 0.0   | FEM   | 1.49 (1.07–2.07)                  |         |
| C vs A         | 21  | 52.7  | REM   | 1.10 (0.97–1.26)                  | .29     |
| HWE: yes       | 17  | 49.9  | REM   | 1.07 (0.97–1.18)                  | .29     |
| Distribution: North | 8   | 51.1  | REM   | 1.22 (1.01–1.47)                  | .08     |
| Distribution: South | 13  | 51.9  | REM   | 1.02 (0.85–1.23)                  |         |

OR = odds ratio, CI = confidence interval, MTHFR = 5,10-methylentetrahydrofolate reductase; URPL = unexplained recurrent pregnancy loss; No. = number; HWE = Hardy–Weinberg equilibrium.

* REM: random effects model; FEM: fixed effects model.
† P value for north vs south.
individuals carrying the wild-type homozygous genotype (677CC/1298AA) (Figure 2).

### 3.4. Relationship between MTHFR C677T or MTHFR A1298C geographical distribution and the risk of URPL

We also performed a sub-group analysis stratified by north and south China (Table 5 and 6). For MTHFR C677T, twenty-five studies with 3725 URPL cases and 4217 controls were included in the north China and twenty-five studies with 2952 URPL cases and 3894 controls were included in the south China. Significant associations between MTHFR C677T and URPL risk were found in both north and south China in all the models. However, no significant difference was observed in all the genotype groups between north and south China in the risk of URPL (Table 5). For MTHFR A1298C, eight studies with 1686 URPL cases and 1528 controls were included in the north China and thirteen studies with 1753 URPL cases and 1627 controls were included in the south China. Sub-group analyzed results still showed significant association in the recessive and homozygous models of MTHFR A1298C polymorphism in north and south China. In addition, we also found a significant association in the allele model (C vs A; OR 1.18) and C1298A genotype occurrence more significantly in the URPL population than in the control group, while eight studies showed the opposite trend.

### 3.5. Sensitivity analysis

We conducted sensitivity analysis to ascertain the primary origin of the heterogeneity. Through sensitivity analysis, the present study showed that no individual study had affected the pooled ORs (see Figure S1 and S2). The results were consistent with previous meta-analysis results among Chinese population reported by Chen et al and Ren et al, and among Asian population reported by Parveen et al, Cao et al, and Wu et al. For MTHFR A1298C, a total of 21 studies involving 3439 URPL cases and 3155 controls were included. A significant association between MTHFR A1298C mutations (recessive [CC vs AC+AA]; OR 1.55; 95% CI 1.25–1.93) and homozygous [CC vs AA; OR 1.53; 95% CI 1.22–1.91] models) and URPL was observed. Among the related 21 studies, two studies showed that the MTHFR 1298CC genotype occurred more significantly frequent in the URPL population than in the control group and eleven studies described the same trend, while eight studies showed the opposite trend. Our present meta-analysis showed significantly positive results, which were similar with the meta-analyzed ones reported by Yang et al and Zhang et al, but were inconsistent with previous meta-analyzed results reported by Chen et al, Cao et al, and Wu et al. We hypothesize that part of the reasons for these controversial results might be related to negative results occupied high weights and different ethnic background in their meta-analyses.

### 4. Discussion

In our meta-analysis, we collected 50 studies with 6677 URPL cases and 8111 controls to investigate the association between MTHFR C677T gene polymorphism and the risk of URPL. The results revealed that all the C677T mutations of MTHFR were significantly associated with the risk of URPL in the Chinese population. The MTHFR 677 T allele and TT genotype may increase the risk of URPL. These results were expected because all the individual studies included in our meta-analysis presented these trends in their populations. The results were also consistent with previous meta-analysis results among Chinese population reported by Chen et al and Ren et al, and among Asian population reported by Parveen et al, Cao et al, and Wu et al. For MTHFR A1298C, a total of 21 studies involving 3439 URPL cases and 3155 controls were included. A significant association between MTHFR A1298C mutations (recessive [CC vs AC+AA]; OR 1.55; 95% CI 1.25–1.93) and homozygous [CC vs AA; OR 1.53; 95% CI 1.22–1.91] models) and URPL was observed. Among the related 21 studies, two studies showed that the MTHFR 1298CC genotype occurred more significantly frequent in the URPL population than in the control group and eleven studies described the same trend, while eight studies showed the opposite trend. Our present meta-analysis showed significantly positive results, which were similar with the meta-analyzed ones reported by Yang et al and Zhang et al, but were inconsistent with previous meta-analyzed results reported by Chen et al, Cao et al, and Wu et al. We hypothesize that part of the reasons for these controversial results might be related to negative results occupied high weights and different ethnic background in their meta-analyses.
no significant association among Chinese women (OR 2.71; 95% CI 0.84–8.70). Further analysis revealed that among the five studies, one study presented MTHFR C677T and MTHFR A1298C joint mutation more frequent in the control than in the URPL group and has the highest weight (24.52%). And this might be the reason why we did not get statistically significant result.

The association of geographical distributions of the MTHFR C677T and MTHFR A1298C gene polymorphisms with URPL was not clear among Chinese population. Our results showed that geographical distributions of the MTHFR C677T or MTHFR A1298C gene polymorphisms were not associated with URPL.

Some potential limitations of this meta-analysis should be addressed. One of the limitations was high heterogeneity. It influenced the interpretation of the meta-analysis result. Sample size, racial difference, and deviations of allele distributions from the HWE law played important roles. After excluded non HWE studies, the results still played high heterogeneity in the recessive, homozygous, and additive comparisons in MTHFR C677T group. Our further analysis showed the high heterogeneity was caused by Shang et al, whose pooled OR value and sample size were high. After we removed this study, all the I² values decreased to less than 50%. While for MTHFR A1298C, no significant heterogeneity was found in all the comparisons after remove non HWE articles. The other limitation was publication bias. Positive results interested only by researchers and journals might be the possible reason for publication bias. However, in our present study, deviation from HWE law was an indication of potential publication bias. After excluding the non HWE studies, all the P values increased to over.05 in both MTHFR C677T and MTHFR A1298C groups. In addition, for the association between MTHFR C677T and MTHFR A1298C joint mutation and the risk of URPL, only five studies were involved, so heterogeneity and publication bias were not investigated. Therefore, additional studies were needed to reevaluate the risk of MTHFR C677T and MTHFR A1298C joint mutation in URPL. In addition, although we performed a broad search in five different databases to find studies for inclusion criteria, it is impossible to confirm that all available studies were included, which may exhibit another limitation of our meta-analysis.

In conclusion, this meta-analysis suggested that MTHFR C677T allele and 677TT genotype and MTHFR 1298CC genotype may be risk factors for the development of URPL. Moreover, geographical distribution of the MTHFR C677T or MTHFR A1298C gene polymorphisms was not associated with the risk of URPL for Chinese women. We suggested that MTHFR C677T and MTHFR A1298C mutants should be tested in Chinese pregnant women.

Author contributions

Data curation: Xiaoying Wang, Qiang Sun, Baiqian Xing.
Investigation: Zhong-dong Li.
Methodology: Gengzhu Wang, Xiaoying Wang, Qiang Sun.
Resources: Zhaohui Lin, Zhikun Xun.
Software: Gengzhu Wang, Qiang Sun, Baiqian Xing.
Supervision: Zhong-dong Li.
Validation: Zhaohui Lin, Xiaoying Wang, Zhikun Xun, Baiqian Xing.
Writing – original draft: Gengzhu Wang.
Writing – review & editing: Zhaohui Lin, Zhong-dong Li.

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