


## ORIGINAL ARTICLE

# Association of *MTHFR* C677T polymorphism and type 2 diabetes mellitus (T2DM) susceptibility

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## Abstract

**Introduction:** Methylenetetrahydrofolate reductase (*MTHFR*) is essential in mediating folate metabolism, and thus plays an important role in diabetes and diabetic complications. *MTHFR* C677T (rs1801133 C>T) polymorphism has been proposed to be linked with type 2 diabetes mellitus (T2DM) susceptibility. However, the conclusions are inconsistent. Therefore, we rechecked their linkage aiming to obtain a more reliable estimation by performing an updated meta-analysis.

**Methods:** We searched electronic databases PubMed, EMBASE, CNKI, and Wanfang to obtain studies updated to October 2019.

**Results:** After carefully screening, we finally incorporated 68 studies with 10,812 cases and 8,745 controls. The genotype frequency of C677T polymorphism was analyzed pooled to generate odds ratios (ORs) and 95% confidence intervals (CIs). Pooled results presented that *MTHFR* C677T polymorphism was significantly associated with T2DM under homozygous (OR = 1.64, 95% CI = 1.39–1.94), heterozygous (OR = 1.38, 95% CI = 1.20–1.59), recessive (OR = 1.41, 95% CI = 1.23–1.61), dominant (OR = 1.47, 95% CI = 1.27–1.70), and allele (OR = 1.37, 95% CI = 1.23–1.52) genetic models. Stratified analysis demonstrated that C677T genotype was associated with T2DM in Asian populations, but not Caucasian and African populations.

**Conclusion:** Our results indicated that *MTHFR* C677T polymorphism confers to T2DM, especially in Asian populations. Much more large-scale case–control studies are needed to strengthen such conclusion in the future.

## KEYWORDS

C677T, meta-analysis, *MTHFR*, polymorphism, T2DM

## 1 | INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major public health problem that not only affects individual life quality, but also increases social economic burden (DeFronzo et al., 2015).

The frequency of T2DM in China is increasing quickly, with estimation of about 380 million T2DM patients by 2025 (van Dieren, Beulens, van der Schouw, Grobbee, & Neal, 2010). The etiology of T2DM remains partly elucidated. Evidences suggest that T2DM is a complex disease caused by

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the combinations of environmental and genetic risk factors (Wareham, Franks, & Harding, 2002; Zeggini et al., 2007).

Previous reports showed that individuals with insufficient intake of folic acid were more likely to have T2DM. Folate is a methyl group donor in the synthesis of intracellular methylation reactions and de novo deoxynucleoside (Blount et al., 1997). When folate deficiency, the DNA stability will be impaired (Duthie, 1999). Methylenetetrahydrofolate reductase (MTHFR) is a folate-metabolizing enzyme that participates in folic acid circulation and DNA synthesis (Friso et al., 2002). MTHFR catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Niclot et al., 2006). Dysfunction or low activity of MTHFR may decrease the level of methyl pool; consequently, it inhibits the successful deoxynucleoside synthesis and intracellular methylation reactions (Rozen, 1997).

The human gene *MTHFR* (OMIM number: 607093) is located on chromosome 1p36.3. Of all the identified SNPs, C677T (Ala222Val, rs1801133 C>T) is one of the most investigated genetic variations (Adinolfi et al., 2005; Liew & Gupta, 2015). The C677T polymorphism is a C to T transition at base pair 677, which results in the amino acid transition from Ala to Val. Such amino acid transition significantly decreases the activity of MTHFR (Weisberg, Tran, Christensen, Sibani, & Rozen, 1998). Recent data suggested that there exist an association between C677T and the susceptibility of T2DM. However, the role of C667T in risk of T2DM was discrepant. Several meta-analyses that were conducted to solve this conflicting role somehow failed. To get a precise estimation, we re-analyzed the role of *MTHFR* C677T on T2DM via including larger eligible investigations.

## 2 | MATERIALS AND METHODS

### 2.1 | Literature search

We carried out a comprehensive literature search in the following databases: PubMed, EMBASE, CNKI, and Wanfang. The searching was updated to October 2019 without any language limitations. The combination of the following search terms was adopted: '*MTHFR* or methylenetetrahydrofolate reductase', and 'polymorphism or polymorphisms or SNP or single nucleotide polymorphism or variant' and 'diabetes or mellitus or diabetes mellitus or T2DM'. To expand the included studies, we also retrieved eligible references from the selected studies. The GenBank reference sequence and version number for the gene is: *MTHFR* (NM\_005957.5).

### 2.2 | Inclusion/exclusion criteria

We set the following criteria when performing the selection work: (a) evaluating the association of *MTHFR* C677T polymorphism with T2DM risk; (b) case-control design; (c) odds

ratios (ORs) and their 95% confidence intervals (CIs) were able to obtain; and (d) reports Hardy-Weinberg equilibrium (HWE). Exclusion criteria were as follows: (a) reviews or meta-analyses; (b) case-only studies or case reports; and (c) duplicate publications.

### 2.3 | Data extraction

We arranged three authors to handle data extraction: two authors to extract data independently and one author to resolve the disagreement. The following data were selectively extracted from each study: first author's surname, year of publication, country, ethnicity, genotyping methods, and genotypic distribution. The stratification analysis was conducted by ethnicity (Asians, Caucasians, and Africans) and HWE (HWE <0.05 and HWE >0.05).

### 2.4 | Statistical methods

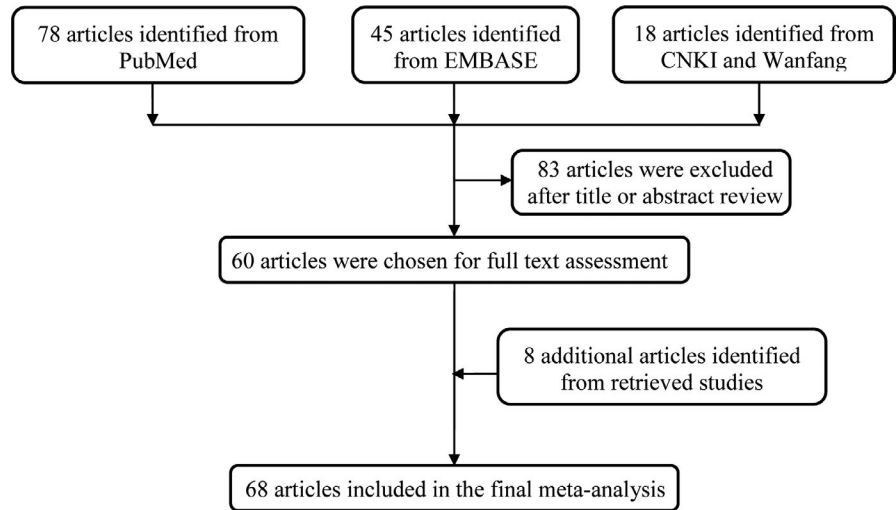
STATA 11.0 software (Stata Corporation) was adopted to conduct the current meta-analysis. We first used Chi-square test to check whether the genotype frequency of C677T among the controls was in HWE. After that, we determined the relationship between *MTHFR* C677T polymorphism and T2DM risk by calculating pooled ORs with the corresponding 95% CIs. We totally used five genetic models: homozygote model (TT vs. CC), heterozygote model (CT vs. CC), recessive model (TT vs. CT/CC), dominant model (CT/TT vs. CC), and allele model (T vs. C) to detect such relationship. Stratification analyses were also taken by ethnicity (Asian, Caucasian, and African) and HWE (HWE <0.05 and HWE >0.05), aiming to detect the source of heterogeneity. We carried out Chi-square-based Q statistic test and inconsistency index statistics ( $I^2$ ) to calculate heterogeneity between study results. If the studies were homogeneous (with  $p^{het} < .10$  or  $I^2 > 50\%$ ), the random-effects model (the DerSimonian and Laird method) was chosen. Otherwise, ORs were calculated using the fixed-effects model (the Mantel-Haenszel method). Sensitivity analysis was performed to assess the strength of the conclusion, by sequentially excluding each study at a time. Begg's funnel plot and Egger's linear regression test were conducted to assess publication bias. We also conducted quality assessment to detect the quality of each study using the quality assessment criteria (He et al., 2014). All the statistics were two-sided.  $p < .05$  was considered as significant.

## 3 | RESULTS

### 3.1 | Study search

General process of publication selection was graphically shown in Figure 1. Initial retrieval from PubMed and EMBASE databases got a total of 78 and 45 potentially

FIGURE 1 Search flow diagram



relevant published records, respectively. We also obtained 18 articles from Chinese databases CNKI and Wanfang. After titles and abstracts screening, 81 nonrelevant records were excluded. The remaining 60 articles and eight additional articles identified from retrieved studies were included in the final meta-analysis (Al-Harbi et al., 2015; Al-Salihi, Ajeena, Al-Kashwan, & Al-Lebban, 2016; Zidan, El Mougy, Moustafa, El attar, & Mohamed, 2019; Benrahma et al., 2012; Bluthner et al., 1999; Cao, Huang, Mao, & Gao, 2005; Chang et al., 2011; Chen, Ning, Zhu, Li, & Shi, 2004; Chen et al., 2010; P. Chen, Pan, Sun, Bai, & Fu, 2008; Dai & Yu, 2012; El Hajj Chehadeh et al., 2016; Eroglu et al., 2007; Errera et al., 2006; Fekih-Mrissa et al., 2017; Fujita et al., 1999; Guo, Pan, Chu, Guo, & Sun, 2005; Guo et al., 2002; Hu, Zhang, Fang, Qin, & Liu, 2009; Hu, Gan, Li, & Bi, 2001; Jimenez-Ramirez et al., 2017; Ksiazek, Bednarek-Skublewska, & Buraczynska, 2004; Lin, Wang, & Liu, 2009; Liu et al., 2014; Luo, Yan, Li, Cheng, & Song, 2007; Luo, Yan, Ma, Cheng, & Song, 2008; Mao, Gao, Qin, & Shi, 2004; Mehri et al., 2010; Mei, Chen, & Zheng, 2012; Mtiraoui et al., 2007; Neugebauer, Baba, & Watanabe, 1998; Nithya et al., 2017; Odawara & Yamashita, 1999; Pirozzi et al., 2018; Qiu, 2009; Rahimi et al., 2009; Ramanathan, Harichandana, Kannan, Elumalai, & Sfd, 2019; Raza, Abbas, Siddiqi, & Mahdi, 2017; Settin, El-Baz, Ismaeel, Tolba, & Allah, 2015; Shang, Wang, & Liu, 2017; Shi, He, Cheng, Wang, & Liu, 2006; J. Shi, Li, Yu, Chen, & Tao, 2002; Shpichinetsky et al., 2000; Soares et al., 2008; J. Sun, Xu, Xue, Zhu, & Lu, 2005; Sun, Xu, & Zhu, 2001; Sun, Xu, Zhu, & Lu, 2004; Sun, Xu, Lu, & Zhu, 2009; Sun, Chen, et al., 2004; Sun, Wang, Shi, & Yang, 2013; Wang et al., 2017; Wang, Hu, Xiao, & Wan, 2014; Wang, Wang, & Li, 2018; Wang, Wang, Xue, Chen, & Zou, 2001; Wang, Wang, Xue, Cheng, et al., 2001; Wen, Lu, Li, Wu, & Zhang, 2008; Wirta et al., 1998; Xiao, Hu, Shan, Guan, & Ren, 2006; Xu, Zhang, Shan, & Ma, 2003; Yang, Lu, & Pan, 2001; Yilmaz, Agachan, Ergen, Karaalib, & Isbir, 2004; Yoshioka et al., 2004; Yue, Liu, Kang, Hu, & Qiu, 2006; Zhang, Li, Liu, &

Hu, 2007; Zhang, Xiang, Weng, & Li, 2002; Zhang & Liu, 2009; Zhi et al., 2016; Zhou, Li, & Zhang, 2004).

### 3.2 | Study characteristics

The study characteristics of the final selected studies were presented in Table 1. A total of 68 studies with 10,812 cases and 8,745 controls were included in our final meta-analysis. Among these eligible studies, 52 were done on Asians, 11 studies were done on Caucasians, and five studies were done on Africans. As to the HWE, genotype distribution in the controls of 52 studies was agreed with the HWE, and 16 studies were not. We also classified the studies into low-quality studies (48 studies) and high-quality studies (20 studies) by quality score.

### 3.3 | Meta-analysis results

Table 2 and Figure 2 illustrated the main results of the current meta-analysis. We adopted five genetic models to assess the association between *MTHFR* C677T and T2DM: homozygote model TT versus CC, heterozygous model CT versus CC, recessive model TT versus CT/CC, dominant model CT/TT versus CC, and allele model T versus C. There was a significant association between *MTHFR* C677T polymorphism and T2DM under homozygous (OR = 1.64, 95% CI = 1.39–1.94), heterozygous (OR = 1.38, 95% CI = 1.20–1.59), recessive (OR = 1.41, 95% CI = 1.23–1.61), dominant (OR = 1.47, 95% CI = 1.27–1.70), and allele (OR = 1.37, 95% CI = 1.23–1.52) genetic models in a random-effects model.

In the subgroup analysis based on ethnicity, we divided the included studies into three ethnicities: Asian, Caucasian, and African. We found significant association between *MTHFR* C677T genotype and T2DM in Asian populations, under each genetic models homozygous (OR = 1.78, 95% CI = 1.48–2.15), heterozygous (OR = 1.51, 95% CI = 1.33–1.70), recessive (OR = 1.43, 95% CI = 1.23–1.67), dominant

TABLE 1 Characteristics of studies included in the current meta-analysis

| Surname       | Year | Country        | Ethnicity | Genotype method | Case |     |    |       |     | Control |    |       |        |       |
|---------------|------|----------------|-----------|-----------------|------|-----|----|-------|-----|---------|----|-------|--------|-------|
|               |      |                |           |                 | CC   | CT  | TT | Total | CC  | CT      | TT | Total |        |       |
|               |      |                |           |                 | CC   | CT  | TT | Total | CC  | CT      | TT | Total | HWE    | Score |
| Neugebauer    | 1998 | Japan          | Asian     | PCR-RFLP        | 24   | 31  | 12 | 67    | 86  | 43      | 17 | 146   | 0.003  | 6     |
| Wirta V       | 1998 | Finland        | Caucasian | PCR-RFLP        | 46   | 30  | 8  | 84    | 60  | 48      | 7  | 115   | 0.520  | 8     |
| Bluthner      | 1999 | Germany/Poland | Caucasian | PCR-RFLP        | 74   | 50  | 23 | 147   | 67  | 68      | 15 | 150   | 0.708  | 6     |
| Fujita        | 1999 | Japan          | Asian     | PCR-RFLP        | 31   | 57  | 17 | 105   | 20  | 39      | 9  | 68    | 0.142  | 7     |
| Odawara       | 1999 | Japan          | Asian     | PCR-RFLP        | 52   | 65  | 26 | 143   | 38  | 68      | 25 | 131   | 0.578  | 7     |
| Shpichimetsky | 2000 | Israel         | Caucasian | PCR-RFLP        | 23   | 22  | 10 | 55    | 21  | 16      | 6  | 43    | 0.316  | 3     |
| Hu S          | 2001 | China          | Asian     | PCR-RFLP        | 49   | 48  | 16 | 113   | 30  | 24      | 1  | 55    | 0.121  | 5     |
| Sun J         | 2001 | China          | Asian     | PCR-RFLP        | 32   | 33  | 20 | 85    | 10  | 16      | 31 | 57    | 0.008  | 4     |
| Wang L        | 2001 | China          | Asian     | PCR-RFLP        | 52   | 68  | 41 | 161   | 37  | 36      | 12 | 85    | 0.502  | 5     |
| Wang L        | 2001 | China          | Asian     | PCR-RFLP        | 65   | 75  | 39 | 179   | 37  | 38      | 10 | 85    | 0.959  | 7     |
| Yang G        | 2001 | China          | Asian     | PCR-RFLP        | 17   | 27  | 23 | 67    | 26  | 28      | 8  | 62    | 0.914  | 6     |
| Guo Q         | 2002 | China          | Asian     | PCR-RFLP        | 12   | 19  | 22 | 53    | 12  | 11      | 5  | 28    | 0.391  | 7     |
| Shi J         | 2002 | China          | Asian     | PCR-RFLP        | 12   | 31  | 7  | 50    | 22  | 29      | 5  | 56    | 0.291  | 5     |
| Zhang G       | 2002 | China          | Asian     | PCR-RFLP        | 56   | 108 | 34 | 198   | 40  | 49      | 11 | 100   | 0.484  | 7     |
| Xu J          | 2003 | China          | Asian     | PCR-RFLP        | 39   | 54  | 30 | 123   | 20  | 25      | 7  | 52    | 0.853  | 8     |
| Chen A        | 2004 | China          | Asian     | PCR-RFLP        | 24   | 45  | 22 | 91    | 21  | 9       | 5  | 35    | 0.038  | 7     |
| Ksiazek P     | 2004 | Poland         | Caucasian | PCR-RFLP        | 159  | 123 | 44 | 326   | 71  | 83      | 16 | 170   | 0.237  | 10    |
| Mao L         | 2004 | China          | Asian     | PCR-RFLP        | 35   | 37  | 11 | 83    | 26  | 18      | 3  | 47    | 0.960  | 8     |
| Sun J         | 2004 | China          | Asian     | PCR-RFLP        | 102  | 76  | 42 | 220   | 74  | 34      | 22 | 130   | <0.001 | 9     |
| Sun L         | 2004 | China          | Asian     | PCR-RFLP        | 27   | 52  | 27 | 106   | 29  | 18      | 3  | 50    | 0.925  | 7     |
| Yilmaz H      | 2004 | Turkey         | Caucasian | PCR-RFLP        | 121  | 98  | 30 | 249   | 101 | 93      | 20 | 214   | 0.831  | 8     |
| Yoshioka K    | 2004 | Japan          | Asian     | PCR-RFLP        | 21   | 13  | 6  | 40    | 71  | 107     | 29 | 207   | 0.260  | 9     |
| Zhou J        | 2004 | China          | Asian     | PCR-RFLP        | 16   | 78  | 45 | 139   | 8   | 31      | 30 | 69    | 0.998  | 8     |
| Cao H         | 2005 | China          | Asian     | PCR-RFLP        | 14   | 20  | 6  | 40    | 26  | 18      | 3  | 47    | 0.960  | 7     |
| Guo L         | 2005 | China          | Asian     | PCR-RFLP        | 60   | 51  | 50 | 161   | 58  | 34      | 35 | 127   | <0.001 | 8     |
| Sun J         | 2005 | China          | Asian     | PCR-RFLP        | 101  | 78  | 49 | 228   | 63  | 31      | 20 | 114   | <0.001 | 10    |
| Errera FI     | 2006 | Brazil         | Caucasian | PCR-RFLP        | 44   | 41  | 10 | 95    | 36  | 57      | 14 | 107   | 0.244  | 9     |
| Shi C         | 2006 | China          | Asian     | PCR-RFLP        | 108  | 60  | 18 | 186   | 68  | 34      | 7  | 109   | 0.338  | 8     |
| Xiao Y        | 2006 | China          | Asian     | PCR-RFLP        | 16   | 53  | 4  | 73    | 47  | 25      | 1  | 73    | 0.245  | 7     |
| Yue H         | 2006 | China          | Asian     | PCR-RFLP        | 66   | 131 | 55 | 252   | 17  | 11      | 2  | 30    | 0.903  | 8     |

(Continues)

TABLE 1 (Continued)

| Surname             | Year | Country              | Ethnicity | Genotype method | Case |     |    | Control |     |     | HWE | Score |        |       |
|---------------------|------|----------------------|-----------|-----------------|------|-----|----|---------|-----|-----|-----|-------|--------|-------|
|                     |      |                      |           |                 | CC   | CT  | TT | Total   | CC  | CT  |     |       | TT     | Total |
| Eroglu Z            | 2007 | Turkey               | Caucasian | PCR-RFLP        | 51   | 45  | 7  | 103     | 63  | 58  | 7   | 128   | 0.171  | 7     |
| Luo D               | 2007 | China                | Asian     | PCR-RFLP        | 65   | 102 | 26 | 193     | 42  | 35  | 14  | 91    | 0.151  | 7     |
| Mturaoui N          | 2007 | Tunisia              | Caucasian | PCR-RFLP        | 163  | 135 | 62 | 360     | 270 | 94  | 36  | 400   | <0.001 | 12    |
| Zhang C             | 2007 | China                | Asian     | PCR-RFLP        | 28   | 29  | 19 | 76      | 34  | 19  | 12  | 65    | 0.006  | 8     |
| Chen P              | 2008 | China                | Asian     | PCR-RFLP        | 19   | 70  | 27 | 116     | 14  | 73  | 37  | 124   | 0.014  | 9     |
| Luo D               | 2008 | China                | Asian     | PCR-RFLP        | 59   | 63  | 19 | 141     | 43  | 31  | 11  | 85    | 0.166  | 8     |
| Soares AL           | 2008 | Brazil               | Caucasian | PCR-RFLP        | 15   | 8   | 2  | 25      | 9   | 5   | 2   | 16    | 0.363  | 3     |
| Wen J               | 2008 | China                | Asian     | PCR-RFLP        | 22   | 50  | 23 | 95      | 27  | 25  | 5   | 57    | 0.816  | 6     |
| Hu L                | 2009 | China                | Asian     | PCR-RFLP        | 47   | 63  | 49 | 159     | 26  | 17  | 9   | 52    | 0.053  | 7     |
| Liu R               | 2009 | China                | Asian     | PCR-RFLP        | 56   | 36  | 47 | 139     | 93  | 22  | 24  | 139   | <0.001 | 10    |
| Qiu Y               | 2009 | China                | Asian     | PCR-RFLP        | 83   | 68  | 48 | 199     | 53  | 29  | 18  | 100   | <0.001 | 9     |
| Rahimi Z            | 2009 | Iran                 | Asian     | PCR-RFLP        | 33   | 27  | 5  | 65      | 33  | 22  | 4   | 59    | 0.898  | 5     |
| Sun J               | 2009 | China                | Asian     | PCR-RFLP        | 94   | 73  | 48 | 215     | 78  | 38  | 26  | 142   | <0.001 | 10    |
| Zhang Q             | 2009 | China                | Asian     | PCR-RFLP        | 66   | 94  | 66 | 226     | 26  | 17  | 9   | 52    | 0.053  | 8     |
| Chen A              | 2010 | China                | Asian     | PCR-RFLP        | 57   | 74  | 27 | 158     | 34  | 17  | 4   | 55    | 0.373  | 8     |
| Mehri S             | 2010 | Tunisia              | African   | PCR-RFLP        | 50   | 49  | 16 | 115     | 66  | 38  | 12  | 116   | 0.078  | 8     |
| Chang YH            | 2011 | China                | Asian     | PCR             | 1    | 25  | 30 | 56      | 3   | 23  | 36  | 62    | 0.781  | 6     |
| Houda Benrahma      | 2012 | Morocco              | African   | PCR-RFLP        | 160  | 97  | 25 | 282     | 114 | 122 | 26  | 262   | 0.420  | 10    |
| Dai H               | 2012 | China                | Asian     | PCR-RFLP        | 51   | 54  | 15 | 120     | 31  | 27  | 2   | 60    | 0.176  | 8     |
| Mei Q               | 2012 | China                | Asian     | PCR-RFLP        | 17   | 51  | 23 | 91      | 17  | 70  | 37  | 124   | 0.076  | 8     |
| Sun L               | 2013 | China                | Asian     | PCR-RFLP        | 180  | 243 | 48 | 471     | 30  | 42  | 6   | 78    | 0.094  | 11    |
| Liu K               | 2014 | China                | Asian     | PCR-RFLP        | 103  | 54  | 6  | 163     | 54  | 23  | 0   | 77    | 0.123  | 8     |
| Han Wang            | 2014 | China                | Asian     | TaqMan          | 234  | 293 | 66 | 593     | 298 | 312 | 70  | 680   | 0.377  | 12    |
| Al-Harbi EM         | 2015 | Bahrain              | Asian     | PCR-RFLP        | 116  | 43  | 12 | 171     | 135 | 47  | 6   | 188   | 0.449  | 10    |
| Ahmad Settin        | 2015 | Egypt                | African   | PCR-RFLP        | 111  | 65  | 27 | 203     | 156 | 135 | 20  | 311   | 0.195  | 11    |
| Al-Salihi NJ        | 2016 | Iraqi                | Asian     | PCR-RFLP        | 28   | 28  | 5  | 61      | 12  | 10  | 0   | 22    | 0.167  | 4     |
| El Hajj Chehadeh SW | 2016 | United Arab Emirates | Asian     | TaqMan          | 155  | 49  | 5  | 209     | 132 | 27  | 10  | 169   | <0.001 | 10    |
| Xueyuan Zhi         | 2016 | China                | Asian     | TaqMan          | 28   | 86  | 66 | 180     | 76  | 172 | 102 | 350   | 0.826  | 11    |
| Fekih-Mrissa N      | 2017 | Tunisia              | African   | PCR-RFLP        | 56   | 102 | 2  | 160     | 124 | 68  | 8   | 200   | 0.726  | 11    |

(Continues)

TABLE 1 (Continued)

| Surname            | Year | Country     | Ethnicity | Case            |     |     | Control |       |     | Score |    |     |        |     |
|--------------------|------|-------------|-----------|-----------------|-----|-----|---------|-------|-----|-------|----|-----|--------|-----|
|                    |      |             |           | Genotype method | CC  | CT  | TT      | Total | CC  |       | CT | TT  | Total  | HWE |
| Jimenez-Ramirez FJ | 2017 | Puerto Rico | Caucasian | PCR-RFLP        | 72  | 8   | 9       | 89    | 184 | 159   | 57 | 400 | 0.020  | 10  |
| K Nithya           | 2017 | India       | Asian     | PCR-RFLP        | 173 | 25  | 2       | 200   | 94  | 6     | 0  | 100 | 0.757  | 10  |
| Raza ST            | 2017 | India       | Asian     | PCR-RFLP        | 152 | 162 | 65      | 379   | 102 | 52    | 26 | 180 | <0.001 | 11  |
| Shang G            | 2017 | China       | Asian     | PCR-RFLP        | 84  | 106 | 36      | 226   | 66  | 91    | 37 | 194 | 0.573  | 11  |
| Wang D             | 2017 | China       | Asian     | PCR-RFLP        | 69  | 72  | 21      | 162   | 162 | 127   | 13 | 302 | 0.052  | 10  |
| Pirozzi FF         | 2018 | Brazil      | Caucasian | PCR-RFLP        | 17  | 22  | 8       | 47    | 30  | 38    | 9  | 77  | 0.560  | 7   |
| Wang J             | 2018 | China       | Asian     | PCR-RFLP        | 176 | 101 | 103     | 380   | 183 | 70    | 53 | 306 | <0.001 | 11  |
| Ramanathan G       | 2019 | India       | Asian     | PCR-RFLP        | 72  | 71  | 2       | 145   | 81  | 19    | 0  | 100 | 0.293  | 10  |
| Zidan              | 2019 | Egypt       | African   | PCR-RFLP        | 30  | 51  | 39      | 120   | 54  | 6     | 0  | 60  | 0.683  | 9   |

Abbreviations: HWE, Hardy–Weinberg equilibrium; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism.

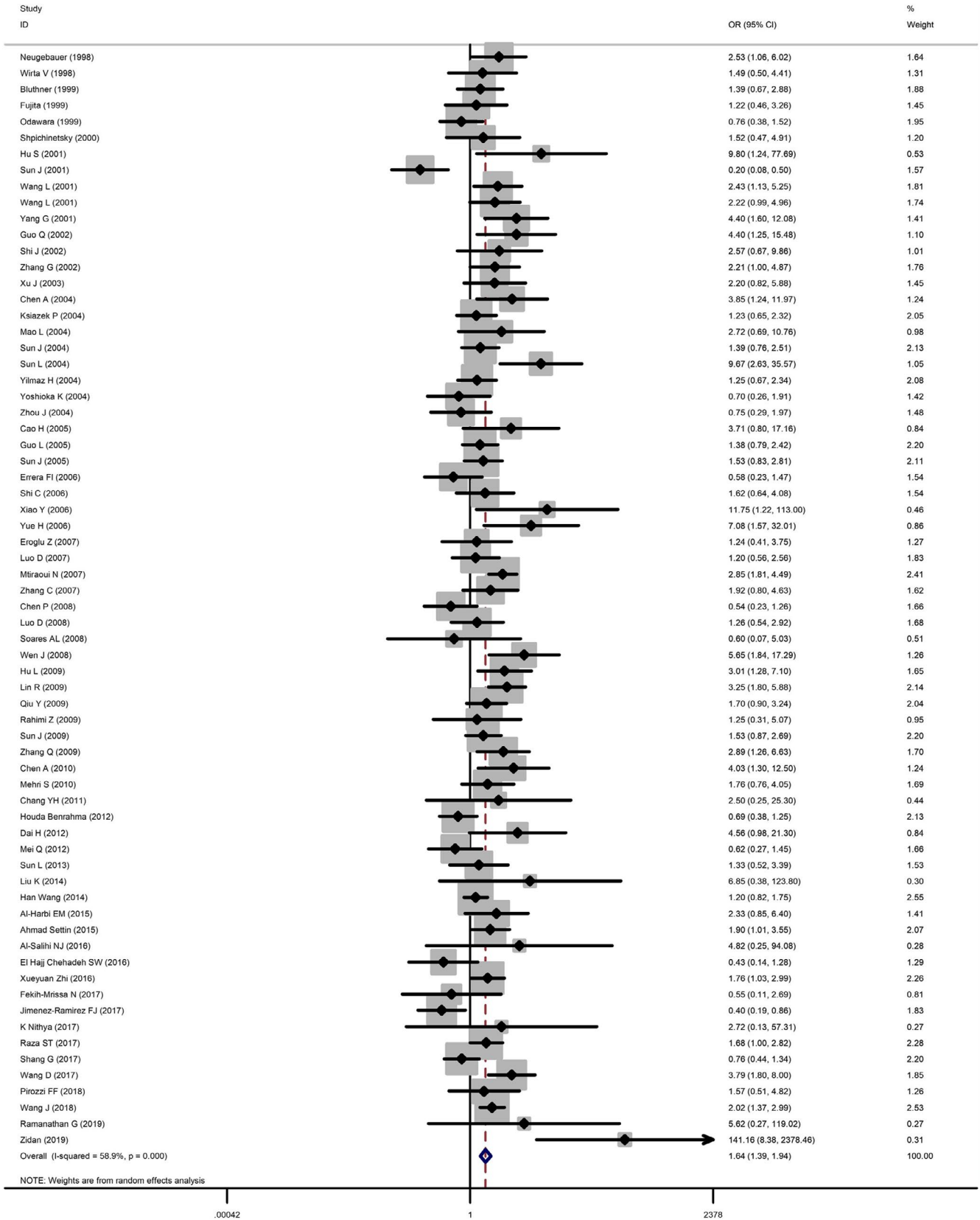
TABLE 2 Meta-analysis of the association between *MTHFR* C677T polymorphism and T2DM susceptibility

| Variables     | No. of studies | Homozygous              |             | Heterozygous            |             | Recessive               |             | Dominant                |             | Allele                  |             |
|---------------|----------------|-------------------------|-------------|-------------------------|-------------|-------------------------|-------------|-------------------------|-------------|-------------------------|-------------|
|               |                | TT versus CC            | OR (95% CI) | CT versus CC            | OR (95% CI) | TT versus CT/CC         | OR (95% CI) | CT/TT versus CC         | OR (95% CI) | T versus C              | OR (95% CI) |
| All           | 68             | <b>1.64 (1.39–1.94)</b> | <.001       | <b>1.38 (1.20–1.59)</b> | <.001       | <b>1.41 (1.23–1.61)</b> | <.001       | <b>1.47 (1.27–1.70)</b> | <.001       | <b>1.37 (1.23–1.52)</b> | <.001       |
| Ethnicity     |                |                         |             |                         |             |                         |             |                         |             |                         |             |
| Asian         | 52             | <b>1.78 (1.48–2.15)</b> | <.001       | <b>1.51 (1.33–1.70)</b> | <.001       | <b>1.43 (1.23–1.67)</b> | <.001       | <b>1.60 (1.40–1.82)</b> | <.001       | <b>1.44 (1.29–1.59)</b> | <.001       |
| Caucasian     | 11             | 1.20 (0.81–1.79)        | .007        | 0.79 (0.52–1.21)        | <.001       | <b>1.43 (1.14–1.79)</b> | 0.457       | 0.87 (0.57–1.32)        | <.001       | 0.97 (0.72–1.32)        | <.001       |
| African       | 5              | 1.70 (0.63–4.57)        | <.001       | 1.88 (0.75–4.74)        | <.001       | 1.45 (0.62–3.39)        | 0.002       | 2.15 (0.86–5.42)        | <.001       | 1.92 (0.98–3.73)        | <.001       |
| HWE           |                |                         |             |                         |             |                         |             |                         |             |                         |             |
| >0.05         | 52             | <b>1.76 (1.45–2.13)</b> | <.001       | <b>1.34 (1.14–1.57)</b> | <.001       | <b>1.50 (1.29–1.75)</b> | 0.006       | <b>1.48 (1.26–1.73)</b> | <.001       | <b>1.39 (1.24–1.56)</b> | <.001       |
| ≤0.05         | 16             | 1.38 (0.99–1.92)        | <.001       | <b>1.48 (1.11–1.99)</b> | <.001       | 1.19 (0.92–1.55)        | <.001       | <b>1.44 (1.07–1.95)</b> | <.001       | <b>1.29 (1.01–1.65)</b> | <.001       |
| Quality score |                |                         |             |                         |             |                         |             |                         |             |                         |             |
| >9            | 20             | <b>1.46 (1.12–1.89)</b> | <.001       | 1.29 (0.99–1.69)        | <.001       | <b>1.37 (1.12–1.68)</b> | 0.003       | <b>1.36 (1.05–1.75)</b> | <.001       | <b>1.30 (1.09–1.56)</b> | <.001       |
| ≤9            | 48             | <b>1.78 (1.43–2.23)</b> | <.001       | <b>1.42 (1.21–1.67)</b> | <.001       | <b>1.45 (1.21–1.73)</b> | 0.001       | <b>1.53 (1.29–1.82)</b> | <.001       | <b>1.40 (1.23–1.60)</b> | <.001       |

Note: The GenBank reference sequence and version number for the gene is: *MTHFR* (NM\_005957.5). Values were in bold if 95% CIs excluded 1 or *p* values less than .05.

Abbreviations: CI, confidence interval; Het, heterogeneity; HWE, Hardy–Weinberg equilibrium; OR, odds ratio; T2DM, type 2 diabetes mellitus.





**FIGURE 2** Forest plot of association between *MTHFR* C677T polymorphism and T2DM under homozygous model. The horizontal lines represent the study-specific ORs and 95% CIs, respectively. The diamond represents the pooled results of OR and 95% CI. CI, confidence interval; OR, odds ratio; T2DM, type 2 diabetes mellitus

(OR = 1.60, 95% CI = 1.40–1.82), and allele (OR = 1.44, 95% CI = 1.29–1.59). However, no relationship was found between *MTHFR* C677T genotype and T2DM in Caucasian and African, except for the recessive model in Caucasian group (OR = 1.43, 95% CI = 1.14–1.79). When stratified by HWE, significant association was also observed in the subgroup of HWE >0.05, under homozygous (OR = 1.76, 95% CI = 1.45–2.13), heterozygous (OR = 1.34, 95% CI = 1.14–1.57), recessive (OR = 1.50, 95% CI = 1.29–1.75), dominant (OR = 1.48, 95% CI = 1.26–1.73), and allele (OR = 1.39, 95% CI = 1.24–1.56) genetic models. Significant association was detected in heterozygous (OR = 1.48, 95% CI = 1.11–1.99), dominant (OR = 1.44, 95% CI = 1.07–1.95), and allele (OR = 1.29, 95% CI = 1.01–1.65) in the subgroup of HWE < 0.05. A subgroup analysis stratified by quality score was also conducted. Significant association was detected in homozygous (OR = 1.46, 95% CI = 1.12–1.89), recessive (OR = 1.37, 95% CI = 1.12–1.68), dominant (OR = 1.36, 95% CI = 1.05–1.75), and allele (OR = 1.30, 95% CI = 1.09–1.56) genetic models, in the subgroup of quality score > 9. Significant association was also detected in homozygous (OR = 1.78, 95% CI = 1.43–2.23), heterozygous (OR = 1.42, 95% CI = 1.21–1.67), recessive (OR = 1.45, 95% CI = 1.21–1.73), dominant (OR = 1.53, 95% CI = 1.29–1.82), and allele (OR = 1.40, 95% CI = 1.23–1.60) genetic models, in the subgroup of quality score ≤ 9.

### 3.4 | Heterogeneity and sensitivity analysis

As shown in Table 1, substantial heterogeneities could be found among all the genetic models ( $p < .001$ ) for the *MTHFR* C677T. Therefore, the random-effect model was used to calculate the pooled ORs and 95% CIs for all the models.

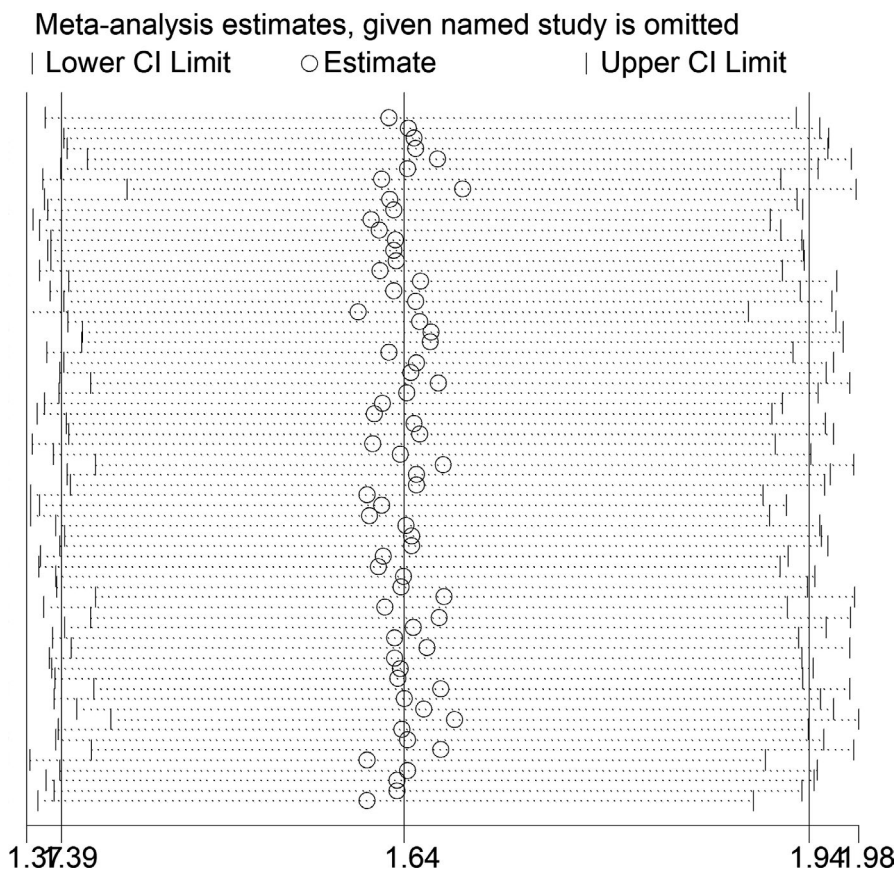
Sensitivity analysis using sequential leave-one-out strategy was carried out to explore the influence of a single study on the pooled ORs. The omission of each study did not impact the recalculated ORs, indicating the credibility and reliability of our results (Figure 3).

### 3.5 | Publication bias

Begg's funnel plot and quantitative Egger's test were adopted to test the publication bias of the current meta-analysis. As indicated by the symmetrical shape of the Begg's funnel plots, no significant publication bias was observed (Figure 4). Moreover, Egger's test also suggested the nonexistence of publication bias among the studies (data not shown).

## 4 | DISCUSSION

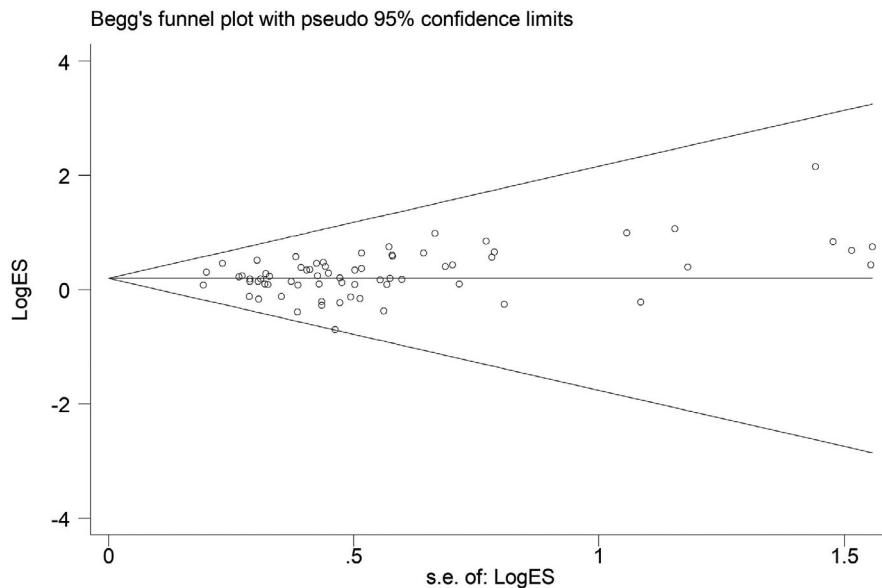
To our knowledge, the current meta-analysis represents the largest and most comprehensive one regarding the relationship between *MTHFR* C677T and T2DM so far. Our



**FIGURE 3** Sensitivity analysis of the association between *MTHFR* C677T polymorphism and T2DM. Each point represents the recalculated OR after deleting a separate study. OR, odds ratio; T2DM, type 2 diabetes mellitus



**FIGURE 4** Funnel plot analysis for assessing publication bias for *MTHFR* C677T polymorphism under homozygous model. Each point represents a separate study for the indicated association



analysis provided strong evidence that *MTHFR* C677T was significantly associated with T2DM, especially in Asians. Sensitivity analysis indicated that there was no significant change in the overall results by removing one study in each turn. Publication bias analysis also showed that the results are convincible.

*MTHFR* C677T is a functional genetic variation that leads to amino acid substitution from alanine to valine (Ueland, Hustad, Schneede, Refsum, & Vollset, 2001). Such amino acid shift was illustrated to compromise the enzyme activity to nearly 50%, compared to the wild-type *MTHFR* enzyme (Weisberg et al., 1998). Although the relationship between T2DM susceptibility and *MTHFR* C677T genotype has been largely investigated, contradictory conclusions still remain. In 2006, no evidence of association was found by F.I.V. Errera et al. between the 677TT genotype of *MTHFR* and T2DM, in Brazilian populations (Errera et al., 2006). In a study conducted in China in 2014, Wang et al. found that C677T in the *MTHFR* may influence the risk of T2DM (Wang et al., 2014). Recently, in a case–control study conducted in the population of Brazilian with 47 T2DM cases and 78 controls by Flavio Fontes Pirozzi et al. (Pirozzi et al., 2018), no correlation was found between the *MTHFR* C677T in the development of T2DM.

Due to the divergent results among single-country studies, several systematic meta-analyses have been undertaken to determine conclusively whether *MTHFR* C677T is associated with the risk of T2DM. In 2013, Chinese academics Zhong, Rodriguez, Yang, and Li (2013) conducted a meta-analysis regarding *MTHFR* C677T and T2DM. Their meta-analysis included 4,855 T2DM patients and 5,242 controls. However, they failed to obtain clear evidence of a significant association of *MTHFR* C677T and T2DM across all 39 studies conducted in 15 countries. They also failed to provide compelling

evidence of an association specifically for African, Asian, or Caucasian populations. Interestingly, Khalid et al. (Al-Rubeaan et al., 2013) observed that there was a significant relationship between *MTHFR* C677T polymorphism and T2DM in Arab population, in 2013. In 2014, Zhu et al. (2014) conducted an updated meta-analysis in Chinese population aiming to better identify the role of C677T polymorphism in T2DM. They included 29 studies with 4,656 T2DM patients and 2,127 controls. They detected a significant relationship between *MTHFR* C677T polymorphism and T2DM in the Chinese Han population.

Genotype frequencies at the C677T locus of *MTHFR* vary widely by ethnicity (Errera et al., 2006; Yilmaz et al., 2004), raising the possibility that any association between this SNP and the risk of T2DM may likewise depend on ethnicity. Thus, we further put our focus on ethnic stratification analysis based on the groups that emerged from our literature searches: African, Asian, and Caucasian. Our analysis provided strong evidence that *MTHFR* C677T was significantly associated with T2DM in Asians, but not in Caucasians or Africans. Thus, it is necessary to identify the role of C677T in different ethnicities.

Several weaknesses should be pointed out before interpreting our conclusion. First, selection bias could not be avoided as only the articles in English and Chinese were analyzed. Other studies written in other languages were unable to include. Second, analyzing one SNP in *MTHFR* was far more enough, as the development of T2DM was associated with multiple SNPs in multiple genes. Third, we also failed to determine the role of other potential influential factors in the initiation of T2DM. These potential influential factors such as life style, environment exposures, and gene–environment interactions were reported to be associated with T2DM. Fourth, it is inevitable to avoid several shortages such as

misclassified genotypes, unwell-matched sources of controls, and inconsistent qualities of the included studies, due to the retrospective nature of meta-analysis. Finally, between-study heterogeneity was found in all comparisons, which may compromise the reliability of conclusion.

## 5 | CONCLUSION

In all, this meta-analysis provides a precise conclusion that *MTHFR* C677T polymorphism was significantly associated with T2DM, especially in Asian populations. Further well-designed, large-scale, and in-depth studies are warranted to check such relationship.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR CONTRIBUTIONS

Y.M. and X.L. conceived the study design and wrote the paper. K.M., L.Z., M.L., and M.Z. performed the selection, collected the data, and performed the statistical analysis. M.G. and G.Q. were responsible for the quality control of data. All authors read and approved the final manuscript.

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