Chin Med Sci J
March 2020

Vol. 35, No. 1 P. 71-84
doi:10.24920/003535

ORIGINAL ARTICLE

# Methylenetrahydrofolate Reductase Gene C677T Polymorphism and Diabetic Retinopathy: a Meta-Analysis

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**Key words:** methylenetrahydrofolate reductase gene C677T; polymorphism; diabetic retinopathy; meta-analysis

**Objective** To investigate the association between the methylenetetrahydrofolate reductase gene C677T (*MTHFR* C677T) polymorphism and diabetic retinopathy (DR).

**Methods** A total of 6971 subjects including 2707 DR patients and 4264 controls from 23 studies were enrolled in the study. A random-effects model was applied to estimate the overall effects and the stratified effects of the *MTHFR* C677T polymorphism on the risk of DR, and study quality was also assessed.

**Results** Strong associations were observed between the *MTHFR* C677T polymorphism and DR. The carries of *MTHFR* C677T were more likely to be found in the DR group in relative to the healthy control group with odds ratio 1.68, 2.55, and 2.31 respectively in allele contrast model (T *vs.* C, 95%*CI*: 1.29-2.18, $P<0.001$, $I^2=78.4\%$), homozygous model (TT *vs.* CC, 95%*CI*: 1.70-3.83, $P=0.008$, $I^2=54.4\%$) and dominant model (TT+CT *vs.* CC, 95%*CI*: 1.62-3.29, $P<0.001$, $I^2=74.7\%$). This association can also be found in contrast to the Ncd (non-complicated diabetic mellitus) group (allele contrast, $OR=1.50$, 95%*CI*: 1.07-2.11, $P=0.032$, $I^2=62.1\%$; homozygous, $OR=2.39$, 95%*CI*: 1.06-5.38, $P=0.017$, $I^2=66.7\%$; dominant, $OR=1.59$, 95%*CI*: 0.97-2.62, $P=0.056$, $I^2=56.5\%$). For the heterozygous model (CT *vs.* CC), the association was significant in contrast to the healthy control group ($OR=1.46$, 95%*CI*: 1.64-3.69, $P=0$, $I^2=77.3\%$), while in contrast to the Ncd control group the association was not statistically meaningful ($OR=1.38$, 95%*CI*: 0.87-2.18, $P=0.131$, $I^2=43.7\%$). For the recessive model, 1.92-fold increased risk was found only in contrast to the Ncd control group (95%*CI*: 1.07-3.43, $P=0.064$, $I^2=55.0\%$). There was no significant association found in the models in contrast to the DM control group.

**Conclusion** In this meta-analysis, we found an association between the *MTHFR* C677T polymorphism and DR, especially in contrast to the Ncd control group. Further studies are required to establish more definite relationship.

Received June 2, 2019. Published online March 16, 2020.
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This study was supported by the Beijing Natural Science Foundation (7131007).

DIABETIC retinopathy (DR) has increasingly become the most common cause of vision loss in adults with diabetes mellitus globally. The number of people with diabetes mellitus (DM) is expected to 592 million by 2035,[1] of whom 35.8% will suffer from DR.[2] Many factors such as hyperglycemia, long duration of diabetes mellitus, and insulin use may contribute to DR,[3] however, none of which can fully explain the pathophysiology of DR. It is well known that DM is a multifactorial disease influenced by many genetic variants.[4] Methylenetetrahydrofolate reductase gene (MTHFR), locating chromosome 1p36.3, has been identified as a candidate gene for DM. *MTHFR* encodes methylenetetrahydrofolate reductase, which can catalyze methylation of homocysteine to methionine.[5] The *MTHFR* rs1801133 locus point mutation, in which the cytosine (C) is substituted with thymine (T), has been proved to reduce the enzyme activity of MTHFR, thus leading to hyperhomocysteinemia (HHcy).[6-8] High plasma homocysteine participates in the damage to arterial endothelial cells, subsequently leading to vascular diseases, such as atherosclerosis,[9] renal vascular disease,[10] as well as DR.[11-13] In addition, retinal ganglion cell dysfunction and focal vascular leakage have been observed in *MTHFR* deficient mice presenting with HHcy.[14] Various studies have been carried out to verify association of *MTHFR* C677T polymorphism with DR, but the results have been inconsistent. To elucidate this issue clearly, we conducted a meta-analysis including 23 studies (including our own data which have not been published) addressed from 1998 to 2017.[12, 15-36]

## MATERIALS AND METHODS

### Search strategy and selection criteria

This systematic review and meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement.[16]

We selected studies published from Jan 1st, 1998 to Mar 1st, 2018 by searching PubMed, Embase and Cochrane in English and CNKI in Chinese using the following search terms: "(*methylenetrahydrofolate reductase* or *MTHFR*) and (*gene* or *genotype* or *genetic* or *allele*) and (*diabetic* or *diabetes* or *diabetes mellitus* or *T1DM* or *T2DM*) and (*retinopathy* or *microangiopathy*)". When two articles shared the same or part of the same study population, only the one with the larger sample size was included.

### Inclusion/exclusion criteria

We included studies satisfying the following criteria: (1) studies addressing *MTHFR* polymorphism, where each genotype frequency was reported or was provided by authors after being contacted; (2) the control group was made up of DR-free individuals who were healthy or who had other diabetic complications. When the study had both a healthy control group and a control group with other microvascular complications, we divided the study into two parts, which we considered to be different studies in our analysis.

### Extracted information

Two investigators (SC and LYY) independently extracted data from each study that qualified and entered them into separate databases. The following information was extracted: first author's last name, publication year, population ethnicity, study design, baseline characteristics of the study including age, gender, DM duration, DR diagnosis criteria, DM type and the C677T genotype counts. When the integrity of data was not satisfactory, authors were contacted to request the relevant information. Any identified discrepancies were adjudicated by professor Liu, and a consensus was reached.

The Newcastle-Ottawa Scale (NOS) was used to assess the observational studies. Thus, a methodological quality assessment scale was designed (**Table 1**) according to previous studies and polymorphism meta-analyses in other disciplines. Five items were included: the representativeness of cases, source of controls, sample size, quality control of genotyping methods and Hardy-Weinberg equilibrium (HWE). The quality score ranged from 0 to 10, with a high score signifying good study quality.

### Statistical analysis

In our meta-analysis, we used *OR* and 95%*CI* for genetic comparisons between the case and control groups. The following comparisons were performed: allele (C *versus* T), homozygous genotype (TT *versus* CC) and heterozygous genotype (CT *versus* CC), dominant model (TT+CT *versus* CC), and recessive model (TT *versus* CT+CC). HWE was calculated for the control group in each study using a *Chi-square* test. A value of $P<0.05$ indicated a significant departure from HWE. In

**Table 1.** Methodological quality assessment scale

| Criteria | Score |
|---|---|
| Representativeness of cases | |
| DR diagnosed according to ETDRS | 2 |
| DR diagnosed according to other DR criteria | 1.5 |
| DR diagnosed according to doctors' assessments | 1 |
| Not mentioned | 0 |
| Source of controls | |
| Population or community based | 3 |
| Hospital-based DM-free controls | 2 |
| DR-free DM patients without other complications | 1 |
| DR-free DM patients with other complications | 0.5 |
| Not described | 1 |
| Sample size (*n*) | |
| >200 | 2 |
| 100-200 | 1 |
| <100 | 0 |
| Quality control of genotyping methods | |
| Repetition of partial/total tested samples with a different method | 2 |
| Repetition of partial/total tested samples with the same method | 1 |
| Not described | 0 |
| Hardy-Weinberg equilibrium | |
| Hardy-Weinberg equilibrium in control subjects | 1 |
| Hardy-Weinberg disequilibrium in control subjects | 0 |
| Quality scores | 10 |

DR: diabetic retinopathy; ETDRS: Early Treatment Diabetic Retinopathy Study; DM: diabetes mellitus.

addition, stratified analyses were conducted by control group type, ethnicity and DM type.

We set our level of statistical significance to be a *P* value less than 0.5 as determined by *Z*-test. Heterogeneity was evaluated by the *Q* statistic (significance level of $P<0.1$) and the $I^2$ statistic (a statistic greater than 50% was taken as evidence of significant inconsistency). According to the heterogeneity results, either a fixed-effect model or a random-effect model was used to combine values from each study; when the effects were assumed to be heterogeneous, the random-effects model was used, otherwise the fix-effects model was used. A sensitivity analysis was conducted to evaluate the effect of each study on the combined *OR*s by omitting low-quality studies identified through quality assessment. Potential publication bias was assessed by Begg's funnel plots and Egger's regression asymmetry test, and further confirmed by the Trim and Fill method.

All analyses were done using Stata 14.0 and IBM SPSS Statistics 23.0. All *P*-values were two-sided.

## RESULTS

### Description of studies

From the online databases of PubMed, Embase and CNKI, we identified 96 articles, including 40 from PubMed, 40 from Embase and 14 from CNKI. After the duplications were excluded (including articles published both in Chinese and English), there were 52 articles to be screened. According to the title and abstract of the articles, 29 were excluded for focusing on other diseases or DM vascular complications other than DR (for studies focusing on the microvascular complications of DM, if DR was mentioned in the abstract, it was included). We reviewed the full text of the remaining 27 articles, and 5 studies have been excluded. One was focused on a different single nucleotide polymorphism (SNP) for *MTHFR*; 2 studies did not provide the exact number of subjects with each genotype of C677T; and in 2 studies, the case-patient group and the control group both had DR and could not be divided into a DR and non-DR group (**Figure 1**).

There were therefore 22 published studies included in this study.[12, 15-36] Our own data with 262 T2DM cases and 212 healthy controls from a cohort study of the Beijing Desheng Diabetic Eye Study were also included, that has not been published previously. The methods of clinical evaluation and genotyping is listed below, while the basic materials of the 474 patients enrolled are listed in **Table S1**.

The study protocol was approved by the Ethics Committee of Beijing Tongren Hospital (TRECKY200907) and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants before their enrollment. Patients with T2DM were recruited between November 2009 and September 2011 from the Desheng Community of urban Beijing. Diabetes was defined as a self-reported history of physician-diagnosed T2DM treated with insulin, oral hypoglycemic agents, or diet only; or by a fasting plasma glucose concentration of 7.0 mmol/L (126 mg/dl) or more in at least two previous examinations; or a random plasma glucose concentration of ⩾11.1 mmol/L (200 mg/dl). All subjects underwent a standardized evaluation consisting of a questionnaire, ocular and anthropometric ex-

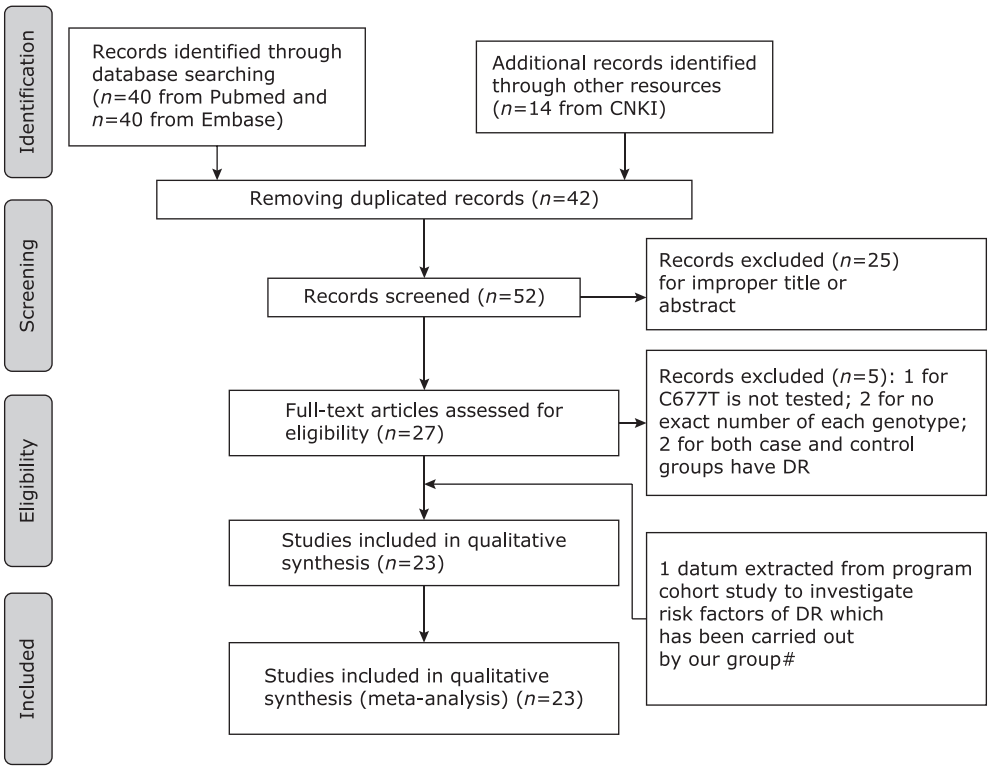


**Figure 1.** Flow chart of the literature search. #: Our own data unpublished.

aminations, and a laboratory test. The questionnaire elicited basic information (age, sex, ethnicity, income, education), lifestyle information (such as smoking and alcohol intake), health status information (such as the use of insulin therapy and any history of systemic diseases), and family history of diseases. Anthropometric parameters included body weight and height, waist and hip circumference, and three measurements, 5 min apart, of blood pressure in a resting state. Body mass index (BMI, $kg/m^2$) was calculated according to the height and weight of the participant, and waist-to-hip ratio was calculated. A comprehensive ophthalmological examination included corrected visual acuity, slit-lamp biomicroscopy, and dilated fundus photography. Seven fields of 30° color fundus photographs with stereoscopic images of the optic disc and macula were taken through the dilated pupils of each patient, using a digital fundus camera (Zeiss Visucam Pro; Oberkochen, Germany).

Blood samples were collected from all participants and stored at −80°C before DNA extraction. Genomic DNA was extracted from venous blood leukocytes using a genomic DNA extraction and purification kit (TIANamp Swab DNA Kit; Tiangen Biotech, Beijing, China). Study participants were genotyped for the SNPs using Sequenom MassARRAY technology (Bioyong Technologies, Beijing, China). All DNA samples passing initial quality checks were plated at a concentration of ⩾5 ng/μl for processing on the platform.

The basic characteristics extracted from the total of 23 studies are shown in **Table 2**. The frequencies of the *MTHFR* C677T polymorphism alleles and genotype prevalences are shown in **Table 3**. Of the 23 studies, 16 are from East Asia (12 from China, 4 from Japan), 2 from West Asia (Turkey), 2 from Europe (1 from Denmark and 1 from Poland), 3 from the America (1 from Canada, 1 from the United States, 1 from Brazil). Among them there is 1 study focusing on T1DM, and 2 focusing on both T1DM and T2DM. The others were all aimed at T2DM.

**Summary statistics**

From all 23 studies, there were 6971 subjects comprising 2707 DR patients and 4264 controls included in this meta-analysis. HWE was satisfied in the distribution of the *MRHFR* C677T polymorphism among 18 studies, while 6 were not consisted with HWE due to genotyping errors and/or population stratification (**Table 2**). We later performed a sensitivity analysis to detect the disequilibrium source. The overall frequency

**Table 2.** Basic extracted characteristics of the investigated studies

| References | Year of publication | Race | Case | | | | | Control | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sample size (*n*) | Age (yrs) | DM duration (yrs) | Definition of case | DR diagnosis | Sample size (*n*) | Age (yrs) | DM[§] duration (yrs) | Defination of control | DM type | HWE* | MAF* | NOS | Quality assessment |
| Beata *et al*.[12] | 2017 | European | 64 | 62.8±9.7 | 16.97±9.2 | DR with DF | ETDRS | 50 | 65.7±9.7 | 17.1±9.48 | DM | T2DM | 0.64 | 0.73 | 5 | 4.5 |
| Najiba *et al*.[16] | 2017 | American | 44 | 50.4±12.92 (all) | 8.98±6.9 | DR | NM | 200 | 50.5±12.77 | NA | healthy | T2DM | 0.72 | 0.21 | 5 | 5 |
| Xing *et al*.[17] | 2016 | Chinese | 76 | NM | NM | DR with/without DN | OC | 56 | no | NA | healthy | T2DM | 0.009 | 0.32 | 6 | 4.5 |
| Wei *et al*.[18] | 2012 | Chinese | 61 | 59.3±12.7 | 6(median) | DR | OC | 64 | 58.3±14.1 | 4(median) | Ncd | T2DM | 0.254 | 0.258 | 6 | 4.5 |
| Sun *et al*.[19] | 2014 | Chinese | 176 | 62.38±8.15 | 16.36±6.47 | DR | DA | 241 | 62.95±8.71 | 12.82±6.1 | DM | T2DM | 0.99 | 0.624 | 6 | 4.5 |
| Guo *et al*.[20] | 2002 | Chinese | 52 | 54.63±12.04 | 4.5 (3.0-8.0) | DR | OC | 28 | 56.57±10.75 | NA | healthy | T2DM | 0.39 | 0.375 | 5 | 4.5 |
| Guo *et al*.[20] | 2002 | Chinese | 52 | 54.63±12.04 | 4.5 (3.0-8.0) | DR | OC | 52 | 55.17±6.87 | 15(median) | Ncd | T2DM | 0.43 | 0.45 | 5 | 4.5 |
| Wang *et al*.[21] | 2001 | Chinese | 62 | 62.5±8.08 | 8.29±6.39 | DR | OC | 117 | 59.42±14.87 | 7.28±5.8 | DM | T2DM | 0.68 | 0.3 | 7 | 4 |
| Wang *et al*.[21] | 2001 | Chinese | 62 | 62.5±8.08 | 8.29±6.39 | DR | OC | 85 | 41.83±17.1 | NA | healthy | T2DM | 0 | 0.73 | 7 | 3.5 |
| Yang *et al*.[22] | 2001 | Chinese | 60 | 50.7±12.1 | <5 | DR | DA | 102 | 48±8.2 | >10 | Ncd | T2DM | 0.17 | 0.41 | 6 | 4 |
| Yang *et al*.[22] | 2001 | Chinese | 60 | 50.7±12.1 | <5 | DR | DA | 62 | 52.6±14.9 | NA | healthy | T2DM | 0.91 | 0.35 | 6 | 5 |
| Sun *et al*.[23] | 2003 | Chinese | 110 | 55.6±6.7 | <5 | DR | OC | 98 | 54.7±7.1 | >10 | DM | T2DM | 0 | 0.33 | 7 | 4 |
| Sun *et al*.[23] | 2003 | Chinese | 110 | 55.6±6.7 | <5 | DR | OC | 57 | 42.3±6.1 | NA | healthy | T2DM | 0 | 0.31 | 7 | 4.5 |
| Huang *et al*.[24] | 2005 | Chinese | 50 | NM | NM | DR (72% with DN) | OC | 47 | no | NA | healthy | T2DM | 0.96 | 0.25 | 5 | 4.5 |
| Yi *et al*.[25] | 2005 | Chinese | 245 | 56.53±10.45 | 5.9±4.8 | DR (27% with protein urine) | OC | 65 | no | NA | healthy | T2DM | 0.01 | 0.31 | 4 | 3.5 |

**Continued Table 2.** Basic extracted characteristics of the investigated studies

| References | Year of publication | Race | Case | | | | | Control | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sample size (*n*) | Age (yrs) | DM duration (yrs) | Definition of case | DR diagnosis | Sample size (*n*) | Age (yrs) | DM[§] duration (yrs) | Defination of control | DM type | HWE[*] | MAF[*] | NOS | Quality assessment |
| Liu *et al*.[26] | 2006 | Chinese | 44 | 51.9±7.5 | NM | DR | DA | 84 | 54.0±13.2 | NA | healthy | T2DM | 0.01 | 0.25 | 6 | 4 |
| Ren *et al*.[27] | 2011 | Chinese | 219 | 59.95±10.55 | 11(median) | DR | DA | 294 | 58.52±12.26 | 7 (median) | DM | T2DM | 0.23 | 0.41 | 6 | 4.5 |
| Santos *et al*.[28] | 2003 | American | 99 | 58.7±12(all) | 14.9 (median) | DR | OC | 111 | 58.7±12 (all) | 6.6 (median) | DM | T2DM | 0.98 | 0.39 | 5 | 5 |
| Errara *et al*.[29] | 2003 | American | 46 | 55.43±15.33 | 18±8.67 | DR(NPDR81, PDR60) | OC | 106 | 66.11±7.06 | NA | healthy | T1DM | 0.24 | 0.39 | 4 | 5.5 |
| Errara *et al*.[29] | 2003 | American | 95 | 55.43±15.33 | 18±8.67 | DR(NPDR81, PDR60) | OC | 106 | 66.11±7.06 | NA | healthy | T2DM | 0.24 | 0.39 | 4 | 6.5 |
| Maeda *et al*.[30] | 2008 | Japanese | 75 | NM | NM | DR | OC | 115 | NM | NM | DM | T2DM | 0.06 | 0.35 | 5 | 4 |
| Yigit *et al*.[31] | 2013 | West Asian | 230 | 57.15±10.58 | 7.73±6.006 | DPN(81DR+DN, 129DN) | ETDRS | 282 | 55.55±8.14 | NA | healthy | T1DM+T2DM | 0.46 | 0.19 | 6 | 6 |
| Yosioka *et al*.[32] | 2003 | Japanese | 98 | 60 (median) | 11.7 (median) | DR(52NPDR, 46PDR) | NM | 268 | 60 | 11.7 | Ncd | T2DM | 0.46 | 0.38 | 4 | 4 |
| Maeda *et al*.[33] | 2003 | Japanese | 51 | NM | NM | DR(33NPDR) | OC | 105 | NM | NM | Ncd | T2DM | 0.06 | 0.37 | 4 | 4 |
| Neugebauer *et al*.[34] | 1998 | Japanese | 67 | 57-61 | 14-16 | DR with DN | NM | 146 | 39-43 | NA | healthy | T2DM | 0.003 | 0.26 | 6 | 4 |
| Lauszus *et al*.[35] | 2001 | European | 112 | NM | NM | DR(T1DM pregnant) | OC | 1084 | NA | NA | healthy | T2DM | 0.53 | 0.29 | 4 | 6 |
| Ukinc *et al*.[36] | 2009 | West Asian | 25 | 52.7±9.9 (all) | 7.6±6.2 (all) | DR | OC | 27 | 52.7±9.9 (all) | 7.6±6.2 (all) | DM | T1DM | 0.09 | 0.24 | 4 | 2.5 |
| Liu *et al*.[#] | 2017 | Chinese | 262 | 66.69±8.28 | 14.40±6.51 | DR(18.1% with microalbuminuria) | ETDRS | 212 | 65.37±7.46 | 14.32±6.11 | DM | T2DM | 0.34 | 0.57 | 8 | 8 |

§: Control with diabetes mellitus and other complications; HWE: Hardy-Weinberg equilibrium; MAF: minor allele frequency; NOS: The Newcastle-Ottawa scale; DF: diabetic foot; DN: diabetic nephropathy; NM: not mentioned; OC: diagnosed according to other criteria; DA: diagnosed according to doctors' assessment; Ncd: non-complicated diabetic mellitus; NA: not applicable; NPOR: non-proliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy; [#]Our own data unpublished; [*]HWE and MAF was calculated in the control group.

of the minor D allele was 0.49 in the cases and 0.36 in the controls. For each study, the MAF in the controls was 0.37 (0.19-0.73). The quality of studies was evaluated using the NOS method and a methodological quality assessment scale. A study was defined as high quality when the scores from two scales were greater than 5 (NOS) and 4 (quality assessment scale) separately.

### *MTHFR* C677T polymorphism and DR

As is shown in **Table 4**, the overall analysis found a significant association between DR and *MTHFR* C677T in all genetic models: allele contrast (C *versus* T, *OR*=1.52, 95%*CI*: 1.27-1.83, *P*=0, $I^2$=80.2%); heterozygous (CT *versus* CC, *OR*=1.81, 95%*CI*: 1.40-2.35, *P*=0, $I^2$=73.5%); homozygous (TT *versus* CC, *OR*=2.27, 95%*CI*: 1.62-3.18, *P*=0, $I^2$=72.3%); dominant model (TT+CT *versus* CC, *OR*=1.86, 95%*CI*: 1.45-2.39, *P*=0, $I^2$=75.0%); recessive model (TT *versus* CT+CC, *OR*=1.55, 95%*CI*: 1.16-2.07, *P*=0, $I^2$=73.7%).

As the included studies had different control groups, which may generate heterogeneity, we divided the analysis into three parts categorized by control group: a healthy control group, a Ncd group (DM without microvascular complications), and a DM group (DM patients with other microvascular complications or not mentioned). From **Table 4**, we can see that heterogeneity was decreased and more precise associations were preserved.

Strong associations were observed between

**Table 3.** The allele/genotype prevalences of *MTHFR* C677T polymorphism of the investigated studies (number of cases)

| References | Year of publication | CC | | CT | | TT | | C | | T | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Case | Control | Case | Control | Case | Control | Case | Control | Case | Control |
| Beata *et al*.[12] | 2017 | 6 | 3 | 30 | 21 | 28 | 26 | 42 | 27 | 86 | 73 |
| Najiba *et al*.[16] | 2017 | 8 | 124 | 36 | 68 | 0 | 8 | 52 | 316 | 36 | 84 |
| Xing *et al*.[17] | 2016 | 17 | 30 | 40 | 16 | 19 | 10 | 74 | 76 | 78 | 36 |
| Wei *et al*.[18] | 2012 | 33 | 37 | 25 | 21 | 3 | 6 | 91 | 95 | 31 | 33 |
| Sun *et al*.[19] | 2014 | 28 | 34 | 88 | 113 | 60 | 94 | 144 | 181 | 208 | 301 |
| Guo *et al*.[20] | 2002 | 5 | 12 | 23 | 11 | 24 | 5 | 33 | 35 | 71 | 21 |
| Guo *et al*.[20] | 2002 | 5 | 17 | 23 | 23 | 24 | 12 | 33 | 57 | 71 | 47 |
| Wang *et al*.[21] | 2001 | 8 | 57 | 27 | 48 | 27 | 12 | 43 | 162 | 81 | 72 |
| Wang *et al*.[21] | 2001 | 8 | 38 | 27 | 10 | 27 | 112 | 43 | 86 | 81 | 234 |
| Yang *et al*.[22] | 2001 | 8 | 32 | 33 | 56 | 19 | 14 | 49 | 120 | 71 | 84 |
| Yang *et al*.[22] | 2001 | 8 | 26 | 33 | 28 | 19 | 8 | 49 | 80 | 71 | 44 |
| Sun *et al*.[23] | 2003 | 33 | 51 | 46 | 29 | 31 | 18 | 112 | 131 | 108 | 65 |
| Sun *et al*.[23] | 2003 | 33 | 31 | 46 | 16 | 31 | 10 | 112 | 78 | 108 | 36 |
| Huang *et al*.[24] | 2005 | 17 | 26 | 25 | 18 | 8 | 3 | 59 | 41 | 70 | 24 |
| Yi *et al*.[25] | 2005 | 68 | 35 | 110 | 19 | 71 | 11 | 246 | 89 | 252 | 41 |
| Liu *et al*.[26] | 2006 | 18 | 47 | 16 | 25 | 10 | 12 | 52 | 119 | 36 | 49 |
| Ren *et al*.[27] | 2011 | 26 | 77 | 78 | 95 | 57 | 41 | 130 | 249 | 192 | 177 |
| Santos *et al*.[28] | 2003 | 34 | 41 | 53 | 53 | 12 | 17 | 121 | 135 | 77 | 87 |
| Errara *et al*.[29] | 2003 | 17 | 36 | 25 | 57 | 4 | 14 | 59 | 129 | 33 | 85 |
| Errara *et al*.[29] | 2003 | 44 | 36 | 41 | 57 | 10 | 14 | 129 | 129 | 61 | 85 |
| Maeda *et al*.[30] | 2008 | 31 | 43 | 28 | 62 | 16 | 10 | 90 | 148 | 60 | 82 |
| Yigit *et al*.[31] | 2013 | 38 | 180 | 30 | 93 | 13 | 9 | 106 | 453 | 56 | 111 |
| Yosioka *et al*.[32] | 2003 | 33 | 100 | 50 | 132 | 15 | 36 | 116 | 332 | 80 | 204 |
| Maeda *et al*.[33] | 2003 | 18 | 37 | 20 | 58 | 13 | 10 | 56 | 132 | 46 | 78 |
| Neugebauer *et al*.[34] | 1998 | 24 | 86 | 31 | 43 | 12 | 17 | 79 | 215 | 55 | 77 |
| Lauszus *et al*.[35] | 2001 | 47 | 542 | 57 | 455 | 8 | 87 | 151 | 1539 | 73 | 629 |
| Ukinc *et al*.[36] | 2009 | 14 | 14 | 11 | 13 | 0 | 0 | 39 | 41 | 11 | 13 |
| Liu *et al*.# | 2017 | 59 | 42 | 118 | 97 | 85 | 73 | 236 | 183 | 288 | 243 |

*MTHFR*: methylenetetrahydrofolate reductase gene; #Our own data unpublished.

the *MTHFR* C677T polymorphism and DR. The carries of *MTHFR* C677T were more likely to be found in the DR group in relative to the healthy control group with odds ratio 1.68, 2.55, and 2.31 respectively in allele contrast model (95%*CI*: 1.29-2.18, *P*<0.001, $I^2$=78.4%), homozygous model (95%*CI*: 1.70-3.83, *P*=0.008, $I^2$=54.4%) and dominant model (95%*CI*: 1.62-3.29, *P*<0.001, $I^2$=74.7%). This association can also be found in contrast to the Ncd (non-complicated diabetic mellitus) group (allele contrast, *OR*=1.50, 95%*CI*: 1.07-2.11, *P*=0.032, $I^2$=62.1%; homozygous, *OR*=2.39, 95%*CI*: 1.06-5.38, *P*=0.017, $I^2$=66.7%; dominant, *OR*=1.59, 95%*CI*: 0.97-2.62, *P*=0.056, $I^2$=56.5%). For the heterozygous model, the association was significant in contrast to the healthy control group (*OR*=2.46, 95%*CI*: 1.64-3.69, *P*=0, $I^2$=77.3%), while in contrast to the Ncd control group the association was not statistical meaningful (*OR*=1.38, 95%*CI*: 0.87-2.18, *P*=0.131, $I^2$=43.7%). For the recessive model, 1.92-fold increased risk was found only in contrast to the Ncd control group (95%*CI*: 1.07-3.43, *P*=0.064, $I^2$=55.0%). There was no significant association found in the models in contrast to DM control group (**Table 4, Figure 2**)

**Results of stratified analysis**

Further analyses were performed by stratfying the included studies with ethnicity and DM type, which would decrease the heterogeneity in the different genetic models (**Table 5**).

In contrast to the healthy control group, different effects were found in Asian subjects *versus* non-Asian subjects. Among Asians, strong associations were enhanced (allele contrast, *OR*=1.93, 95%*CI*: 1.43-2.61, *P*=0.001, $I^2$=69.2%; heterozygous, *OR*=3.22, 95%*CI*: 2.30-4.51, *P*=0.163, $I^2$=31.9%; homozygous, *OR*=3.09, 95%*CI*: 2.08-4.60, *P*=0.170, $I^2$=31.1%; dominant, *OR*=2.90, 95%*CI*: 2.27-3.70, *P*=0.598, $I^2$=0%).

As for DM type, since there were only 3 studies focusing on non-T2DM, we only estimated the association between the T2DM group and controls, which showed a magnified association between the DR and healthy control group (allele contrast, *OR*=1.81, 95%*CI*: 1.31-2.49; heterozygous, *OR*=2.82, 95%*CI*: 1.78-4.48; homozygous, *OR*=2.70, 95%*CI*: 1.78-

**Table 4.** Main results of the association between *MTHFR* C677T polymorphism and DR

| Groups | Genetic models | No. of studies (All/Sensitivity) | *OR* (95%*CI*) | *P* | $I^2$(%) | $OR_{se}$(95%*CI*) | $P_{se}$ | $I^2_{se}$(%) |
|---|---|---|---|---|---|---|---|---|
| Overall | Allele (T *vs*. C) | 28/21 | 1.52 (1.27-1.83) | 0 | 80.2 | 1.46 (1.18-1.82) | 0 | 81.8 |
| | Heterozygous (CT *vs*. CC) | 28/21 | 1.81 (1.40-2.35) | 0 | 73.5 | 1.49 (1.12-1.97) | 0 | 70.1 |
| | Homozygous (TT *vs*. CC) | 27/20 | 2.27 (1.62-3.18) | 0 | 72.3 | 2.21 (1.41-3.48) | 0 | 78.2 |
| | Dominant model (TT+CT *vs*. CC) | 28/21 | 1.86 (1.45-2.39) | 0 | 75.0 | 1.65 (1.22-2.24) | 0 | 77.2 |
| | Recessive model (TT *vs*. CT+CC) | 27/20 | 1.55 (1.16-2.07) | 0 | 73.7 | 1.65 (1.17-2.32) | 0 | 74.5 |
| Healthy control | Allele (T *vs*. C) | 14/11 | 1.68 (1.29-2.18) | 0 | 78.4 | 1.84 (1.38-2.46) | 0 | 73.9 |
| | Heterozygous (CT *vs*. CC) | 14/11 | 2.46 (1.64-3.69) | 0 | 77.3 | 2.27 (1.42-3.63) | 0 | 75.8 |
| | Homozygous (TT *vs*. CC) | 14/11 | 2.55 (1.70-3.83) | 0.008 | 54.4 | 3.02 (1.90-4.80) | 0.036 | 48.3 |
| | Dominant model (TT+CT *vs*. CC) | 14/11 | 2.31 (1.62-3.29) | 0 | 74.7 | 2.43 (1.53-3.84) | 0 | 78.0 |
| | Recessive model (TT *vs*. CT+CC) | 14/11 | 1.49 (0.94-2.37) | 0 | 72.3 | 1.87 (1.23-2.83) | 0.042 | 47.1 |
| Ncd control | Allele (T *vs*. C) | 5/3 | 1.50 (1.07-2.11) | 0.032 | 62.1 | 1.76 (1.03-3.03) | 0.041 | 68.7 |
| | Heterozygous (CT *vs*. CC) | 5/3 | 1.38 (0.87-2.18) | 0.131 | 43.7 | 1.95 (1.16-3.28) | 0.356 | 3.1 |
| | Homozygous (TT *vs*. CC) | 5/3 | 2.39 (1.06-5.38) | 0.017 | 66.7 | 2.47 (1.05-5.84) | 0.069 | 62.6 |
| | Dominant model (TT+CT *vs*. CC) | 5/3 | 1.59 (0.97-2.62) | 0.056 | 56.5 | 2.33 (1.03-5.28) | 0.071 | 62.2 |
| | Recessive model (TT *vs*. CT+CC) | 5/3 | 1.92 (1.07-3.43) | 0.064 | 55.0 | 1.93 (0.79-4.69) | 0.042 | 47.1 |
| DM control | Allele (T *vs*. C) | 9/7 | 1.32 (0.93-1.88) | 0 | 86.6 | 1.17 (0.83-1.66) | 0 | 84.1 |
| | Heterozygous (CT *vs*. CC) | 9/7 | 1.31 (0.87-1.97) | 0.001 | 68.7 | 1.20 (0.79-1.82) | 0.005 | 67.2 |
| | Homozygous (TT *vs*. CC) | 9/7 | 1.83 (0.91-3.69) | 0 | 85.6 | 1.39 (0.77-2.52) | 0 | 78.0 |
| | Dominant model (TT+CT *vs*. CC) | 9/7 | 1.42 (0.89-2.28) | 0 | 79.4 | 1.25 (0.80-1.96) | 0 | 75.3 |
| | Recessive model (TT *vs*. CT+CC) | 9/7 | 1.49 (0.91-2.44) | 0 | 82.3 | 1.22 (0.82-1.83) | 0.002 | 71.6 |

-: not applicable; *OR*: odds ratio; $OR_{se}$, $P_{se}$, and $I^2_{se}$: *SE* (Standard error of mean) of *OR*, *P* value and $I^2$.

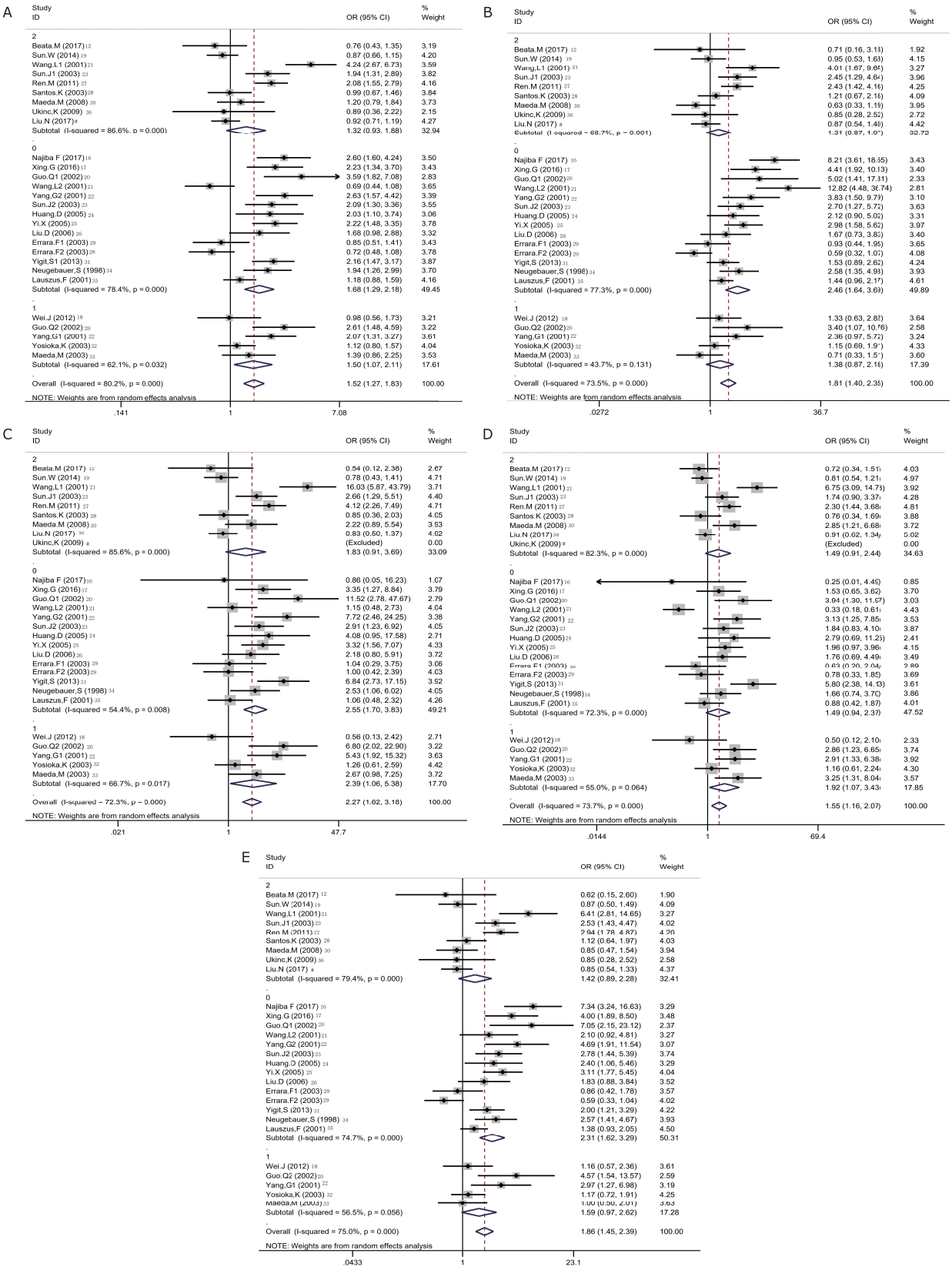


**Figure 2.** Subgroup analysis of associations between *MTHFR* C677T polymorphism and DR by dividing the studied based on the control group categories (0 in these pictures represents the studies having healthy control group, 1 represents the studies having Ncd control group, 2 represents the studies having DM control group. A for allele contrast, B for CT *vs*. CC, C for TT *vs*. CC, D for TT+CT *vs*. CC, E for TT *vs*. CT+CC. #: Our own data unpublished.

4.09; dominant, *OR*=2.75, 95%*CI*: 1.77-4.27). For the recessive model, there was no significant association between the DR and all stratified control groups. (**Table 5**)

As for the DM control group, all stratified analyses were consistent with our original conclusions. Since those in the Ncd group were all Asian and all had T2DM, we did not include the Ncd group in our stratified analyses.

**Sensitivity analysis**

We performed two sensitivity analyses by HWE and study quality. Our sensitivity analysis for the HWE was done in the overall study and did not alter our results. Our sensitivity analysis by study quality affected our findings that in heterozygous genotype model, the association between the Ncd and DR groups was enhanced. Since there were only five studies included in the Ncd group and we excluded two studies in our sensitivity analysis, this changed result has high uncertainty. No changed associations in other genetic models after removing low-quality studies were observed, and the heterogeneity was greatly decreased among Asians when in contrast to the healthy control group.

**Heterogeneity and publication bias**

Heterogeneity was greatly decreased in the stratified analyses and the sensitivity analyses discussed above. We have found that control group type, ethnicity and DM type were large sources of heterogeneity.

Potential publication bias was detected by Egger regression asymmetry test and Begg's adjusted rank correlation test, shown in **Table 6**. Furthermore, we have used the Trim and Fill method to evaluate the publication bias which was found in the heterozygous genetic model and the dominant genetic model, and the result did not show significant publication bias.

## DISCUSSION

In this meta-analysis, the impact of *MTHFR* C667T polymorphism on DR has been evaluated in different genetic models, including allele contrast model, heterozygous model, homozygous model, recessive model and dominant model.[37] Strong associations have been found for each genotype.

The association between the *MTHFR* C677T polymorphism and DR has been detected by several studies in the past decades. These studies have been performed in different regions and using different experimental designs, which resulted in differing conclusions. These differences may have stemmed from different ethnicity, study design, and so on. In some studies, the DR group was divided into proliferative DR and non-proliferative DR,[31, 36] while in others the separation remains unclear.[18, 20-30, 32-35] Other microvascular complications may also affect results, as patients with diabetic nephropathy may yield a stronger association between the *MTHFR* mutation and DR.[22, 26, 33] Ethnicity as well as DM type, may also generate inconsistency, since the studies which considered *MTHFR* gene mutation as susceptibility gene, were all carried out with Asians and with patients of T2DM.[17, 18, 21, 22, 24, 25, 27, 30, 32, 33, 36]

In our research, subgroup analysis by differing the included studies according to the control groups has achieved more precise conclusions. It is worth mentioning that no significant association was found between DR and *MTHFR* C677T variation when the comparison was in contrast to the DM controls, and in contrast to the Ncd group, however, we found that in the homozygous model (TT *versus* CC) and recessive model (TT *versus* CT+CC) *MTHFR* C677T and DR showed strong associations, which indicates an individual with the mutated base pair in homozygous status may have the strongest risk for DR. There are some reasons we consider to explain the phenomenon. First, long-term DM has been thought to be the risk factor of DR development, while some patients with long period of DM actually do not acquire DR, which is defined as the Ncd group in our study. SNPs are thought to be one of the constituent part of the genetic model in polygenetic diseases, our results indicate the accumulation of homozygous mutated SNPs may contribute to incidence of DR in some certain DM group. Second, DR, as the most common microvascular complication of DM, is likely to have the same pathogenesis with other microvascular complications,[38] due to the limitation resources of the specific complications in each study, the association between *MTHFR* genotype and DR in the DM control group may be confounded for the underlying connection.

Furthermore, we have performed stratified analyses and sensitivity analyses by ethnicity, DM type, quality assessment and HWE evaluation.[37] In our stratified analyses, a stronger association between DR and *MTHFR* C677T polymorphism in Asians was found. While the non-Asian stratum was from a variety of regions, since each region had fewer than five studies,

**Table 5.** Associations of *MTHFR* C677T polymorphism and DR in the enrolled studies stratified by ethnicity and DM type

| Groups | | Genetic models | No. of studies (All/Sensitivity) | *OR*(95%*CI*) | *P* | $I^2$(%) | $OR_{se}$(95%*CI*) | $P_{se}$ | $I^2_{se}$(%) |
|---|---|---|---|---|---|---|---|---|---|
| Healthy control | Asian | Allele (T *vs*. C) | 9/7 | 1.93 (1.43-2.61) | 0.001 | 69.2 | 2.18 (1.79-2.65) | 0.701 | 0 |
| | | Heterozygous (CT *vs*. CC) | 9/7 | 3.22 (2.30-4.51) | 0.163 | 31.9 | 2.80 (2.05-3.83) | 0.630 | 0 |
| | | Homozygous (TT *vs*. CC) | 9/7 | 3.09 (2.08-4.60) | 0.170 | 31.1 | 3.55 (2.38-5.29) | 0.405 | 2.7 |
| | | Dominant model (TT+CT *vs*. CC) | 9/7 | 2.90 (2.27-3.70) | 0.598 | 0 | 2.96 (2.22-3.94) | 0.448 | 0 |
| | | Recessive model (TT *vs*. CT+CC) | 9/7 | 1.68 (0.96-2.95) | 0 | 74.1 | 2.08 (1.47-2.96) | 0.782 | 0 |
| | Non-Asian | Allele (T *vs*. C) | 5/4 | 1.32 (0.83-2.09) | 0 | 84.4 | 1.36 (0.72-2.58) | 0 | 88.0 |
| | | Heterozygous (CT *vs*. CC) | 5/4 | 1.53 (0.77-3.06) | 0 | 85.4 | 1.58 (0.58-4.29) | 0 | 89.0 |
| | | Homozygous (TT *vs*. CC) | 5/4 | 1.60 (0.67-3.83) | 0.015 | 67.8 | 1.80 (0.56-5.83) | 0.014 | 71.9 |
| | | Dominant model (TT+CT *vs*. CC) | 5/4 | 1.54 (0.78-3.04) | 0 | 85.9 | 1.61 (0.61-4.29) | 0 | 89.5 |
| | | Recessive model (TT *vs*. CT+CC) | 5/4 | 1.13 (0.43-2.91) | 0.003 | 74.9 | 1.16 (0.31-4.34) | 0.002 | 79.7 |
| DM control | Asian | Allele (T *vs*. C) | 7/5 | 1.49 (0.98-2.26) | 0 | 88.9 | 1.30 (0.89-1.90) | 0 | 85.7 |
| | | Heterozygous (CT *vs*. CC) | 7/5 | 1.38 (0.84-2.28) | 0 | 75.9 | 1.25 (0.73-2.14) | 0.001 | 77.5 |
| | | Homozygous (TT *vs*. CC) | 7/5 | 2.39 (1.05-5.45) | 0 | 88.5 | 1.70 (0.84-3.44) | 0 | 83.4 |
| | | Dominant model (TT+CT *vs*. CC) | 7/5 | 1.58 (0.90-2.78) | 0 | 83.7 | 1.36 (0.77-2.39) | 0 | 82.5 |
| | | Recessive model (TT *vs*. CT+CC) | 7/5 | 1.85 (1.03-3.31) | 0 | 85.6 | 1.44 (0.88-2.35) | 0.001 | 77.8 |
| | Non-Asian | Allele (T *vs*. C) | 2/2 | 0.91 (0.66-1.26) | 0.455 | 0 | – | – | – |
| | | Heterozygous (CT *vs*. CC) | 2/2 | 1.12 (0.65-1.95) | 0.523 | 0 | – | – | – |
| | | Homozygous (TT *vs*. CC) | 2/2 | 0.76 (0.36-1.60) | 0.602 | 0 | – | – | – |
| | | Dominant model (TT+CT *vs*. CC) | 2/2 | 1.03 (0.61-1.75) | 0.45 | 0 | – | – | – |
| | | Recessive model (TT *vs*. CT+CC) | 2/2 | 0.74 (0.43-1.37) | 0.913 | 0 | – | – | – |
| DM control | T2DM | Allele (T *vs*. C) | 8/7 | 1.37 (0.94-1.98) | 0 | 88.1 | 1.18 (0.87-1.60) | 0 | 80.9 |
| | | Heterozygous (CT *vs*. CC) | 8/7 | 1.36 (0.88-2.11) | 0.001 | 72.0 | 1.20 (0.79-1.82) | 0.005 | 67.2 |
| | | Homozygous (TT *vs*. CC) | 8/7 | 1.83 (0.91-3.69) | 0 | 85.6 | 1.39 (0.77-2.52) | 0 | 78.0 |
| | | Dominant model (TT+CT *vs*. CC) | 8/7 | 1.49 (0.90-2.47) | 0 | 81.6 | 1.25 (0.80-1.96) | 0 | 75.3 |
| | | Recessive model (TT *vs*. CT+CC) | 8/7 | 1.49 (0.91-2.44) | 0 | 82.3 | 1.22 (0.82-1.83) | 0.002 | 71.6 |
| Healthy control | T2DM | Allele (T *vs*. C) | 11/9 | 1.81 (1.31-2.49) | 0 | 78.8 | 1.97 (1.43-2.71) | 0 | 72.3 |
| | | Heterozygous (CT *vs*. CC) | 11/9 | 2.82 (1.78-4.48) | 0 | 78.5 | 2.67 (1.52-4.70) | 0 | 77.3 |
| | | Homozygous (TT *vs*. CC) | 11/9 | 2.70 (1.78-4.09) | 0.065 | 42.8 | 2.97 (1.83-4.80) | 0.096 | 40.7 |
| | | Dominant model (TT+CT *vs*. CC) | 11/9 | 2.75 (1.77-4.27) | 0 | 74.6 | 2.82 (1.63-4.89) | 0 | 79.2 |
| | | Recessive model (TT *vs*. CT+CC) | 11/9 | 1.48 (0.89-1.51) | 0 | 70.3 | 1.78 (1.25-2.52) | 0.323 | 13.3 |

-: not applicable; *OR*: odds ratio; $OR_{se}$, $P_{se}$, and $I^2_{se}$: *SE* (Standard error of mean) of *OR*, *P* value and $I^2$.

**Table 6.** Results of Begg's test and Egger's test

| Genetic models | Begg's adjusted rank correlation test (*P* value) | Egger's regression asymmetry test (*P* value) |
|---|---|---|
| Allele | 0.221 | 0.089 |
| CT *vs*. CC | 0.048 | 0.031 |
| TT *vs*. CC | 0.288 | 0.142 |
| Dominant model | 0.048 | 0.056 |
| Recessive model | 0.327 | 0.177 |

we did not divide them to smaller groups out of sample size concerns. Therefore, the possibility of a stronger impact of the *MTHFR* polymorphism in each non-Asian region cannot be excluded and this possibility needs a further study.

Heterogeneity was diminished by stratified analyses and sensitivity analyses as described above. The remaining heterogeneity may come from different study designs. Since DR was diagnosed *via* different criteria in different studies, a more specific definition, such as proliferative DR and non-proliferative DR, may reach a more specific conclusion. In this research there were only two studies that reported non-proliferative DR and proliferative DR group status,[31, 36] which limited conclusions from refined stratified analyses.

Additionally, a sensitivity analysis of HWE was performed in the overall study, which did not affect results from previous study.[39] Lastly, publication bias was considered. In this meta-analysis, we have achieved the largest sample size yet to be reported and have been able to analyze more specific subgroups. Wiltshire *et al*.'s research focused only on T1DM.[38] In Niu *et al*.'s research, only 7 studies were included, which did not yield definite conclusions due to the small sample size.[40] Luo *et al*.'s study included 18 studies published from 1997 to 2014, in which heterogeneity was detected by meta-regression, yielding the conclusion that study design was the main source of heterogeneity.[41] Chen *et al*. performed stratified analysis only by different ethnicity.[42] Meanwhile, in our research, heterogeneity was enormously decreased by elaborate subgroup definition. Also, in our meta-analysis, we included studies that provided comparisons between patients and controls from same community, which enhanced our reliability.

Despite the clear strengths of our study, there remain some limitations. First, only published, English/Chinese studies were included which might generate some bias.[41] Second, DR was diagnosed following differing criteria in different studies, thus a more specific definition, such as proliferative DR and non-proliferative DR, may yield more specific results in the future. Third, confounding factors such as cardiovascular and cerebrovascular history, body mass index, duration of DR, and insulin use were not extracted due to the limited data.

More studies designed along epidemiologic principles with matched confounding factors are needed in the future, and meta-analysis is still important for evaluating the effect of the *MTHFR* C677T polymorphism on the risk of DR.

In conclusion, this meta-analysis finds an association between *MTHFR* C677T polymorphism and DR, especially among those in the Ncd control group. Further studies are required to investigate more precisely the relationship of this *MTHFR* polymorphism and DR.

**The Supporting Information** is available free of charge on the CMSJ website at doi: 10.24920/003535.

**Table S1.** Clinical and biochemical markers for the studied groups.

***Conflict of Interests Statement***

*The authors declare no conflict of interests.*

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