Meta-Analysis

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Methylenetetrahydrofolate reductase C677T polymorphism and diabetic retinopathy risk: a meta-analysis of the Chinese population

Wei-hai Xu[1,2], Ying Zhuang[2], Xiao Han[3] and Zhi-lan Yuan[1]

Abstract

Objectives: This study evaluated associations between methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphisms and diabetic retinopathy (DR) susceptibility within the Chinese population.

Methods: Five databases (PubMed, EMBASE, Web of Science, Cochrane Library, Chinese National Knowledge Infrastructure) were used for literature searches of open access articles from inception through April 2017.

Results: Eight publications were identified involving 600 DR cases, 363 healthy controls, and 646 nondiabetic retinopathy (NDR) controls. There was a positive association between *MTHFR* C677T polymorphisms and DR risk within the Chinese population (DR with NDR controls: T vs. C, odds ratio (OR): 2.14, 95% confidence interval (CI): 1.55–2.97; TT vs. CC, OR: 4.19, 95% CI: 2.09–8.41; TT + CT vs. CC, OR: 2.83, 95% CI: 1.86–4.40; TT vs. CC + CT, OR: 2.48, 95% CI: 1.52–4.05. DR with healthy controls: T vs. C, OR: 2.48, 95% CI: 1.99–3.09; TT vs. CC, OR: 4.92, 95% CI: 3.18–7.62; TT + CT vs. CC, OR: 3.22, 95% CI: 2.32–4.48; TT vs. CC + CT, OR: 3.11,

[1]Department of Ophthalmology, the First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu Province, China
[2]Department of Ophthalmology, Binhai County People's Hospital, Yancheng, Jiangsu Province, China
[3]Department of Biochemistry and Molecular Biology, Key Laboratory of Human Functional Genomics of Jiangsu Province, Nanjing Medical University, Nanjing, Jiangsu, China

Corresponding author:
Xiao Han, Department of Biochemistry and Molecular Biology, Key Laboratory of Human Functional Genomics of Jiangsu Province, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu, China, 210029.
Email: hanxiaonjmu@126.com

95% CI: 1.83–5.28). The association was similar in South China and North China, when stratifying by geographic areas.
Conclusion: *MTHFR* C677T polymorphisms increase DR risk within the Chinese population.

Keywords
Methylenetetrahydrofolate reductase, polymorphism, diabetic retinopathy, meta-analysis, Chinese, disease risk, geographic analysis

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Introduction

Diabetic retinopathy (DR) is a major vascular complication that frequently leads to blindness.[1] Worldwide incidences of DR and vision-threatening DR have been estimated to reach 191.0 million and 56.3 million people, respectively, by 2030, due to the increasing prevalence of diabetes.[2] The prevalences of DR in the Chinese general population and in Chinese diabetic patients were recently estimated at 1.3% and 23%, respectively.[3] Previous studies have suggested that genetic factors and environmental factors contribute to the development of DR.[4] The methylenetetrahydrofolate reductase (*MTHFR*) gene, which catalyzes the methylation of homocysteine to methionine,[5] is widely regarded as a candidate gene for risk of diabetes mellitus. A single nucleotide polymorphism in the *MTHFR* gene at nucleotide C677T can destroy its enzyme activity and cause hyperhomocysteinemia.[6] Because of this critical functional influence, it is readily postulated that *MTHFR* C677T polymorphisms contribute to the development of DR, and a number of studies have addressed their role in DR.[7–9] Data supporting a potential relationship between *MTHFR* C677T polymorphisms and risk of DR within the Chinese population remain controversial, likely because of the lower statistical power of individual studies, which use smaller sample sizes than meta-analyses. In addition, the lack of repeatable results may be due to inconsistent genotyping or lifestyle assessments. Therefore, we performed the present meta-analysis to determine the association between the *MTHFR* C677T polymorphisms and risk of DR within the Chinese population, in order to reduce the influence of distinctive genetic backgrounds or lifestyles.

Materials and methods

Identification and selection of studies

Studies that assessed the relationship between the *MTHFR* C677T polymorphisms and the risk of DR, published before April 2017, were considered in this study. Five databases (PubMed, EMBASE, Web of Science, Cochrane Library, and Chinese National Knowledge Infrastructure) were used for literature searches of open access studies. A combination of keywords ("MTHFR" OR "methylenetetrahydrofolate reductase" AND "DR") was used. Additionally, we carefully reviewed the retrieved references to ensure inclusion of the most comprehensive studies.

Inclusion criteria were as follows: (1) studies using a case-control design that

assessed the relationship between the *MTHFR* C677T polymorphisms and risk of DR; (2) studies with sufficient genotype data for DR cases and healthy controls; (3) studies in which all cases and controls were Chinese individuals; and (4) studies in which DR was assessed by fundus photography or fundus fluorescein angiography, performed in accordance with the methods designated by the 3rd National Congress of Ophthalmology in China. Exclusion criteria were as follows: (1) studies that comprised overlapping cohorts; (2) studies in which data could not be extracted; (3) studies that did not use the case-control design; and (4) studies that were abstracts or reviews.

Data extraction

Two investigators screened the potentially relevant studies and extracted the following data: first author's name, publication year, geographic area, types of controls, sample size, and availability of genotype information regarding the *MTHFR* C677T polymorphisms. The types of controls were stratified as healthy controls and nondiabetic retinopathy (NDR) controls. The titles and abstracts were reviewed for each retrieved document, and the full articles were reviewed if the titles and abstracts did not clearly indicate whether the study was appropriate for this meta-analysis. Discrepancies between the two investigators were resolved by discussion.

Statistical analysis

Odds ratios (ORs) and 95% confidence intervals (CIs) were generated for the *MTHFR* C677T polymorphisms and risk of DR. Models of T versus C, TT versus CC, TT versus (TC+CC) and (TT+TC) versus CC were examined with respect to the risk of DR. The heterogeneity of pooled results, as well as the Hardy-Weinberg equilibrium (HWE) in controls, were assessed by the I^2 statistic, based on the Q-test.[10] A random-effects model was applied to estimate the pooled ORs when $P_{heterogeneity} < 0.1$ or $I^2 > 50\%$; otherwise, a fixed-effects model was adopted. The overall statistical significances of ORs were evaluated by Z-test. Both fixed-effects and random-effects models for each pooled OR were computed for sensitivity analysis. All statistical analysis was performed with Stata version 12 (StataCorp LP, College Station, TX, USA); p-values less than 0.05 were considered significant. Additionally, we performed subgroup analysis by geographic area, to assess the relationship between *MTHFR* C677T and risk of DR in specific regions of China.

Results

Research characteristics

Forty-two publications were identified that assessed relationships between *MTHFR* polymorphisms and risk of DR. In total, eight studies[11–18], which met our inclusion criteria, were used in this report. The publication years of the included studies ranged from 2001 to 2012. Figure 1 shows the detailed screening process used in our analysis. Finally, 600 DR cases, 363 healthy controls, and 646 NDR controls were included in the current study. The main characteristics of the eight articles are listed in Table 1.

Meta-analysis results

We compared DR cases with the NDR group. Analysis of primary pooled statistics showed that all polymorphisms of *MTHFR* C677T (T vs. C, OR: 2.14, 95% CI: 1.55–2.97; TT vs. CC, OR: 4.19, 95% CI: 2.09–8.41; TT + CT vs. CC, OR: 2.83, 95% CI: 1.86–4.40; TT vs. CC + CT, OR: 2.48, 95% CI: 1.52–4.05) (Table 2, Figure 2) had a

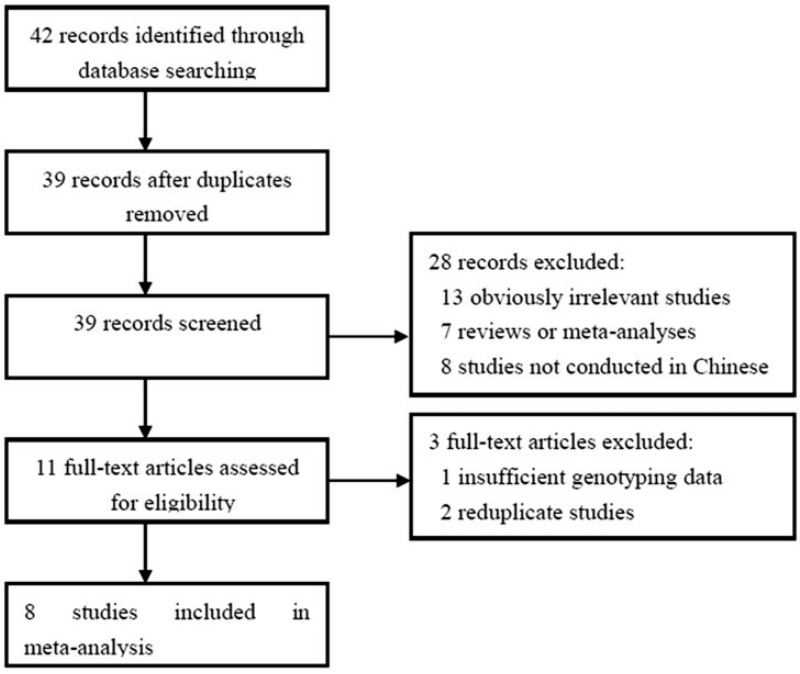

Figure 1. Flow diagram of the literature search.

significantly increased risk of DR. Moreover, subgroup analysis by geographic area showed significantly positive associations among northern Chinese in three analysis models, as well as a significantly positive association among southern Chinese in the (TT+CT vs. CC) model.

We compared DR cases with the healthy group. Analysis of primary pooled statistics showed that all polymorphisms of *MTHFR* C677T (T vs. C, OR: 2.48, 95% CI: 1.99–3.09; TT vs. CC, OR: 4.92, 95% CI: 3.18–7.62; TT + CT vs. CC, OR: 3.22, 95% CI: 2.32–4.48; TT vs. CC + CT, OR: 3.11, 95% CI: 1.83–5.28) could increase the risk of DR; subgroup analysis showed that this risk was also increased specifically in northern Chinese and southern Chinese (Table 3, Figure 3).

Sensitivity analysis

To determine whether the results were stable and robust, sensitivity analyses of both fixed-effects and random-effects models were performed. The results showed that these two models were consistent and stable in each analysis (Table 2, Table 3).

Discussion

Many articles have been published regarding analysis of the relationship between *MTHFR* C677T polymorphisms and risk of DR; however, no comprehensive conclusions have been made. Thus far, three meta-analyses have been published regarding *MTHFR* C677T polymorphisms and risk of DR.[19–21] Nevertheless, the results were inconclusive and inconsistent. Limitations in these three meta-analyses indicated that further studies with larger populations and more rigorous designs are needed.[19–21] Individual studies might yield disparate results, due to the regional and individual differences among populations, as well as

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

Reference	Geographic area	Number of DR cases	Number of healthy controls	Number of NDR controls	Demographic data	Cases			Healthy controls			NDR controls			HWE	
						CC	CT	TT	CC	CT	TT	CC	CT	TT	P_1	P_2
Wang 2001	Guangdong	62	85	117	Sex (M/F): DR 36/26; NDR 63/54; HC 39/46 Age (M/S): DR 62.5/8.1; NDR 59.4/14.9; HC 41.8/17.1	8	27	27	37	38	10	57	48	12	0.959	0.689
Yang 2001	Beijing	60	62	102	Sex (M/F): DR 31/29; NDR 56/46; HC 34/28 Age (M/S): DR 50.7/12.1; NDR 63/8.8; HC 52.6/14.9	8	33	19	26	28	8	32	56	14	0.914	0.178
Guo 2002	Beijing	52	28	52	Sex (M/F): DR 21/31; NDR 25/27; HC 12/16 Age (M/S): DR 54.6/12; NDR 55.2/6.9; HC 56.6/10.8	5	23	24	12	11	5	17	23	12	0.392	0.440
Sun 2003	Hubei	110	57	98	Sex (M/F): DR 64/46; NDR 56/42; HC 34/23 Age (M/S): DR 55.6/6.7; NDR 54.7/7.1; HC 42.3/6.1	33	46	31	31	16	10	51	29	18	0.008	0.001
Huang 2005	Jiangsu	50	47		—	17	25	8	26	18	3				0.961	
Liu 2006	Tianjin	44	84		Sex (M/F): DR 27/17; HC 48/36 Age (M/S): DR 51.9/7.5; HC 54/13.2	18	16	10	47	25	12				0.010	
Ren 2011	Tianjin	161		213	—	26	78	57				77	95	41		0.233
Wei 2012	Guangdong	61		64	Sex (M/F): DR 24/37; NDR 27/37 Age (M/S): DR 59.3/-; NDR 58.3/-	33	25	3				37	21	6		0.254

DR: diabetic retinopathy; HC: healthy controls; NDR: nondiabetic retinopathy; Sex (M/F): Sex (male/female); Age (M/S): Age (mean/standard deviation); P_1: for healthy controls; P_2: for NDR controls

Table 2. Association of the *MTHFR* C677T polymorphism with diabetic retinopathy susceptibility (diabetic retinopathy vs. nondiabetic retinopathy controls).

Analysis model		n	OR_r (95% CI)	OR_f (95% CI)	P_h
T vs. C	Total analysis	6	**2.14 (1.55–2.97)**	**2.16 (1.82–2.56)**	0.006
	South China	3	2.03 (0.95–4.38)	2.16 (1.66–2.81)	0.000
	North China	3	**2.15 (1.72–2.70)**	**2.15 (1.72–2.70)**	0.768
TT vs. CC	Total analysis	6	**4.19 (2.09–8.41)**	**4.14 (2.93–5.86)**	0.006
	South China	3	3.08 (0.59–15.98)	3.56 (2.14–5.90)	0.000
	North China	3	**4.72 (2.93–7.60)**	**4.73 (2.94–7.61)**	0.735
TT vs. CC+CT	Total analysis	6	**2.48 (1.52–4.05)**	**2.49 (1.87–3.30)**	0.028
	South China	3	2.01 (0.56–7.24)	2.44 (1.55–3.84)	0.002
	North China	3	**2.52 (1.75–3.62)**	**2.52 (2.15–3.72)**	0.834
TT+CT vs. CC	Total analysis	6	**2.86 (1.86–4.40)**	**2.83 (1.86–4.40)**	0.056
	South China	3	**2.60 (1.08–6.25)**	**2.57 (1.76–3.75)**	0.008
	North China	3	**3.13 (2.09–4.69)**	**3.13 (2.10–4.69)**	0.765

ORr: Odds ratio for random-effects model; ORf: Odds ratio for fixed-effects model; P_h *P* value for heterogeneity test; North China includes Beijing and Tianjin; South China includes Hubei, Guangdong, and Jiangsu.
Bold values indicate significant results.

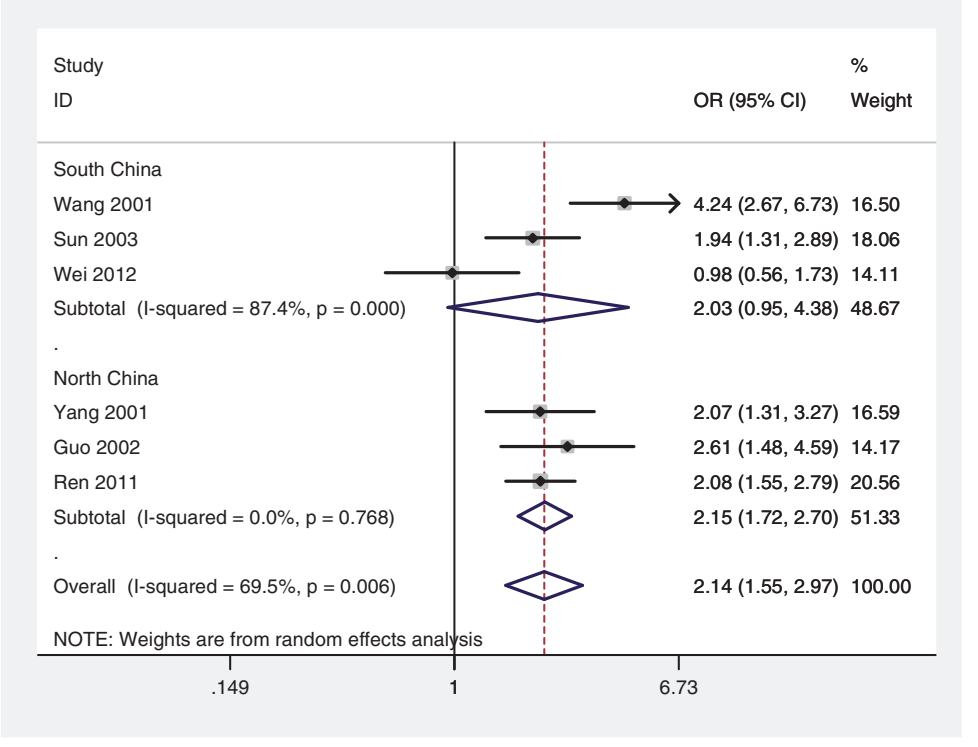

Figure 2. Forest plots of all selected studies regarding the association between *MTHFR* C677T polymorphism and diabetic retinopathy risk within the Chinese population under the allele model (comparison between diabetic retinopathy and nondiabetic retinopathy controls).

Table 3. Association of the *MTHFR* C677T polymorphism with diabetic retinopathy susceptibility (diabetic retinopathy vs. healthy controls).

Analysis model		n	ORr (95% CI)	ORf (95% CI)	P_h
T vs. C	Total analysis	6	**2.48 (1.93–3.19)**	**2.48 (1.99–3.09)**	0.263
	South China	3	**2.53 (1.72–3.71)**	**2.54 (1.89–3.42)**	0.196
	North China	3	**2.43 (1.61–3.68)**	**2.40 (1.74–3.32)**	0.209
TT vs. CC	Total analysis	6	**5.21 (2.81–9.64)**	**4.92 (3.18–7.62)**	0.109
	South China	3	**5.25 (2.03–13.57)**	**4.97 (2.74–9.05)**	0.107
	North China	3	**5.33 (1.92–14.80)**	**4.85 (2.56–9.20)**	0.104
TT vs. CC+CT	Total analysis	6	**2.92 (1.96–4.36)**	**3.11 (1.83–5.28)**	0.379
	South China	3	**3.14 (1.45–6.77)**	**2.75 (1.58–4.78)**	0.148
	North China	3	**2.69 (1.53–4.74)**	**2.93 (2.00–4.30)**	0.510
TT+CT vs. CC	Total analysis	6	**3.25 (2.21–4.77)**	**3.22 (2.32–4.48)**	0.265
	South China	3	**3.15 (2.02–4.90)**	**3.21 (2.08–4.97)**	0.391
	North China	3	**3.59 (1.60–8.06)**	**3.23 (1.95–5.36)**	0.102

ORr: Odds ratio for random-effects model; ORf: Odds ratio for fixed-effects model; P_h *P* value for heterogeneity test; North China includes Beijing and Tianjin; South China includes Hubei, Guangdong, and Jiangsu.
Bold values indicate significant results.

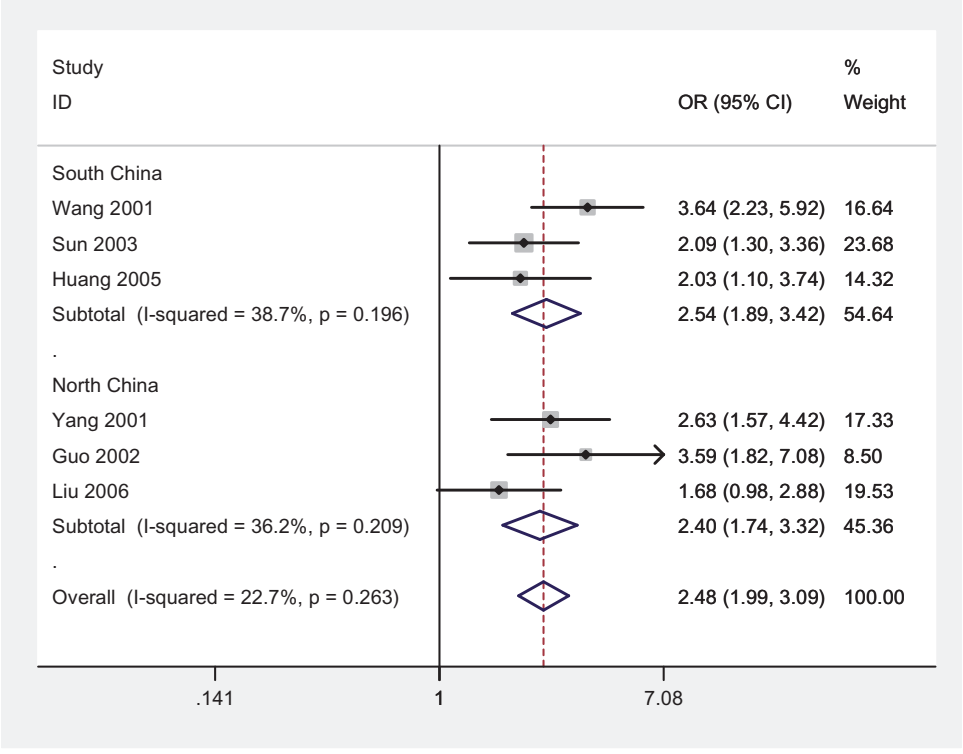

Figure 3. Forest plots of all selected studies regarding the association between *MTHFR* C677T polymorphism and diabetic retinopathy risk within the Chinese population under the allele model (comparison between diabetic retinopathy and healthy controls).

the limited number of cases in each study. Unique lifestyles among diverse ethnic groups may interact differently with particular genetic traits.[22] To reduce the influence of these factors in the overall analysis, we performed this report to further explore the relationship between *MTHFR* C677T polymorphisms and risk of DR within the Chinese population.

The current meta-analysis involved eight studies with 600 DR cases, 363 healthy controls, and 646 NDR controls. The results of this analysis showed a positive relationship between *MTHFR* C677T polymorphisms and risk of DR in overall analyses of DR patients compared with healthy controls or NDR controls. To control for the effects of geographic background on these results, we also performed subgroup analysis with respect to geographic areas; these also showed positive relationships between *MTHFR* C677T polymorphisms and risk of DR in patients in different regions, compared with healthy controls or NDR controls.

There were several limitations in this study. First, we only searched and included openly available articles; therefore, some other unpublished articles or gray literature could have been missed, although they might meet our inclusion criteria. Second, the sample size for our meta-analysis was low, and we could not perform some other subgroup analyses, such as those involving age, exposure time, or smoking habits, owing to the limited data in the original papers. Third, DR is a complex disease involving a variety of factors, both environmental and genetic.[23] However, many of the included studies did not consider some environmental factors, which may influence the risk of DR. Moreover, we did not explore the publication bias in this study, owing to limitations of the funnel plot analysis, which requires a greater number of studies than we had available.

Conclusion

Our study found a positive relationship between *MTHFR* C677T polymorphisms and risk of DR within the Chinese population. However, owing to some limitations in this meta-analysis, further studies with different environmental backgrounds are required to further explore gene-gene and gene-environment influences on *MTHFR* C677T polymorphisms and risk of DR.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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ORCID iD

Xiao Han http://orcid.org/0000-0002-5882-4286

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