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Methylenetetrahydrofolate reductase genetic polymorphism and the risk of diabetic nephropathy in type 2 diabetic patients

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Abstract

Background: As indicated by numerous studies, there exists a relationship between the polymorphism of *methylenetetrahydrofolate reductase (MTHFR)* and susceptibility to diabetic nephropathy (DN) in various populations; nonetheless, the findings remain inconsistent. Therefore, we carried out a meta-analysis to determine the relationship between the *MTHFR* gene polymorphism and DN susceptibility.

Materials and method: Related studies were identified from PubMed, Cochrane Library, EMBASE, and the China National Knowledge Infrastructure database (time period: from building the library to October 2019). The strength of the association was examined using odds ratios (*ORs*) with 95% confidence intervals (*95% CIs*).

Results: The findings illustrated that the *C677T* gene polymorphism was significantly associated with an enhanced susceptibility to DN compared to that with diabetes mellitus in allelic (*OR* = 1.64, *95% CI* = 1.34–2.00, $P < .001$), dominant (*OR* = 1.85, *95% CI* = 1.40–2.46, $P < .001$), codominant (heterozygote: *OR* = 1.67, *95% CI* = 1.27–2.21, $P < .001$; homozygote: *OR* = 2.55, *95% CI* = 1.82–3.57, $P < .001$), and recessive (*OR* = 1.89, *95% CI* = 1.50–2.38, $P < .001$) models of the overall population. Moreover, as compared with the healthy controls, a significantly augmented susceptibility to DN was found in all 5 genetic comparison models (allelic: *OR* = 2.06, *95% CI* = 1.58–2.67, $P < .001$; dominant: *OR* = 2.52, *95% CI* = 1.73–3.69, $P < .001$; codominant: *OR* = 3.78, *95% CI* = 2.50–5.70, $P < .001$; recessive: *OR* = 2.41, *95% CI* = 1.96–2.97, $P < .001$). Furthermore, stratifying data by ethnicity revealed substantially augmented vulnerability to DN in not only Caucasian but also Asian populations.

Conclusion: The present study suggests that the C677T polymorphism was associated with an augmented susceptibility to DN.

Abbreviations: CI = confidence interval, DM = diabetes mellitus, DN = diabetic nephropathy, HWE = Hardy-Weinberg equilibrium, MTHFR = methylenetetrahydrofolate reductase, ORs = odds ratios, T2D = type 2 diabetes.

Keywords: *C677T*, diabetic nephropathy, meta-analysis, methylenetetrahydrofolate reductase, polymorphism

1. Introduction

Type 2 diabetes (T2D) is a highly prevalent chronic disease that affects millions of people globally, accordingly giving rise to substandard health outcomes coupled with elevated healthcare expenditures.[1,2] It is widely accepted that vascular complications constitute the primary causes resulting in diabetes mortality as well as disability. Diabetic nephropathy (DN) is considered among the most common microangiopathic complications of T2D as well as a key cause leading to end-stage renal failure, which affects more than 20% of patients with T2D.[3,4] Robust evidence in studies on the candidate gene relationship and connections suggest the vulnerability of patients to DN.[5] Reportedly, the genetic variants in genes encoding methylenetetrahydrofolate reductase (MTHFR) are likely to confer vulnerability to DN.[6]

MTHFR is a major regulatory enzyme in the metabolism of folate as well as homocysteine.[7] This enzyme catalyzes the remethylation of homocysteine to methionine; additionally, the lack of MTHFR is likely associated with an increase in plasma homocysteine that, consequently, is associated with an augmented susceptibility to vascular diseases, including DN.[8,9] Moreover, the C677T variant frequently found in the gene encoding the folate-metabolizing enzyme MTHFR is considered as the most renowned genetic determinant that influences the localization of folate, accordingly leading to reduced MTHFR activity together with a further elevated homocysteine level.[10]

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HG and M-DX contributed equally to this work.

The authors have no conflicts of interests to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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In 1998, Neugebauer, together with colleagues, proposed for the first time a correlation between the polymorphism of MTHFR C677T with the susceptibility to DN.[11] Consequently, numerous studies have attempted to analyze the effect of the MTHFR C677T polymorphism on DN susceptibility in different populations; nonetheless, no apparent agreement was attained among the results. Consequently, we implemented an updated meta-analysis with current findings to clarify the effects of the MTHFR C677T polymorphism on the susceptibility to DN by using eligible data obtained from the published case-control studies.

2. Materials and methods

2.1. Search strategy

We conducted a computerized literature search in not only PubMed but also in EMBASE, Cochrane Library, and the China National Knowledge Infrastructure database (time period: up to October 2019). MeSH and the title/abstract were used for finding the qualifying case-control studies in accordance with the following keywords:

"methylenetetrahydrofolate reductase OR MTHFR OR C677T" AND "polymorphism* OR mutation* OR variant* OR genotype*" AND "diabetic nephropathy OR diabetes nephropathy." Our study was approved by the Ethics Committee of West Anhui Health Vocational College.

2.2. Inclusion and exclusion criteria

Qualifying studies aligned with the following criteria: the studies

1. estimated the association existing between the polymorphism of MTHFR C677T and the susceptibility to DN;
2. provided sufficient information on C677T genotype frequencies for the determination of odds ratios (*ORs*) with 95% confidence intervals (*95% CIs*) among human individuals with DN; and
3. used a case-control, nested case-control, cross-sectional research design.

Exclusion criteria were as follows:

1. research works without comprehensive genotype data;
2. case studies, reviews, and letters; and
3. duplicate studies.

2.3. Data extraction

Two authors independently extracted the relevant information in accordance with the abovementioned inclusion and exclusion criteria. In addition, the data presented herein were extracted from all included studies: primary author, publication year, country, ethnicity, detection method of genotypes, and the frequency of genotypes among DN patients and controls. Disagreements were resolved by means of discussion between the 2 authors until an agreement was attained.

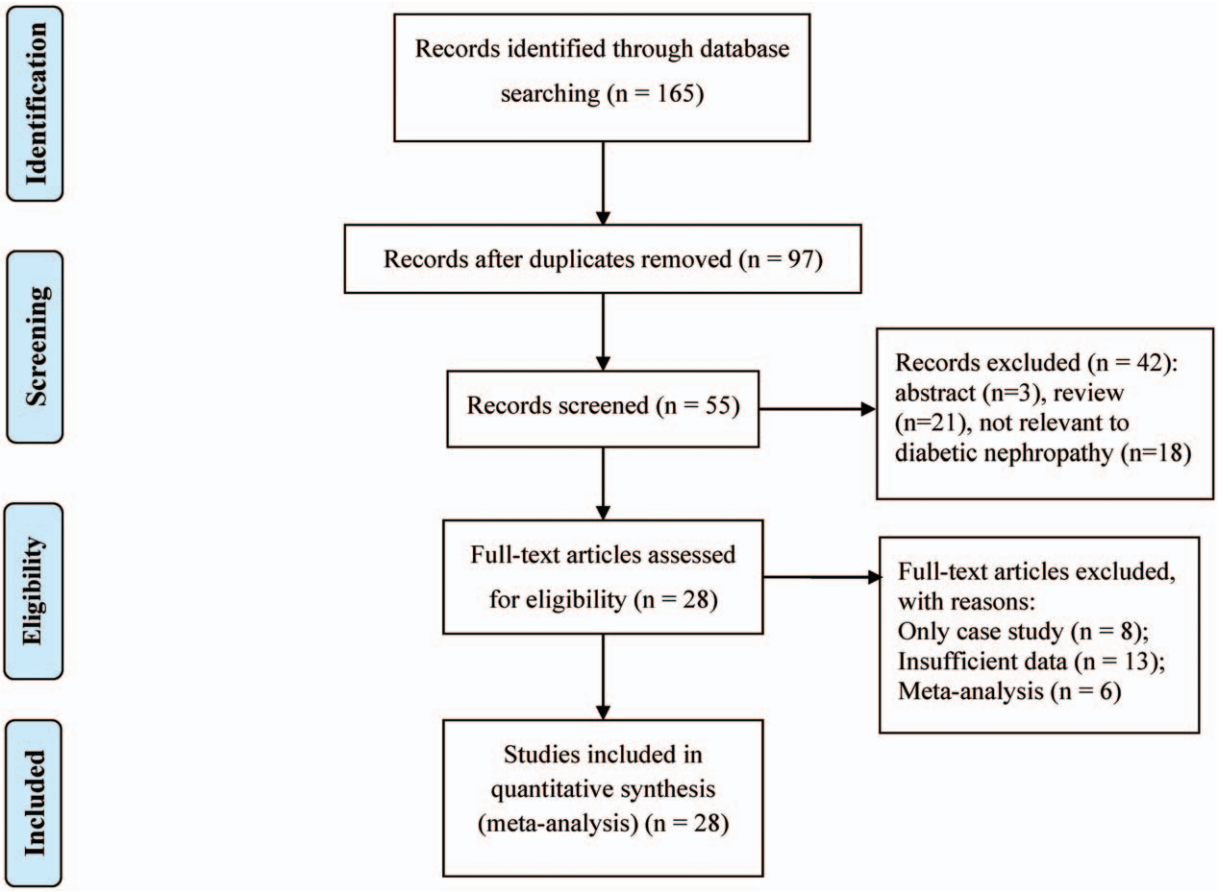

Figure 1. Flow diagram of the literature search.

Table 1

Characteristics of included study.

Author	Year	Country	Ethnicity	Detection method	DN cases			DM controls			Healthy controls			HWE
					CC	CT	TT	CC	CT	TT	CC	CT	TT	
Wang	2001	China	Asian	PCR	20	34	28	32	34	13	37	36	12	0.502
Sun	2001	China	Asian	PCR-RFLP	8	20	11	24	13	9	31	16	10	0.008
Chen	2004	China	Asian	PCR	6	21	14	18	24	8	21	9	5	0.039
Yang	2001	China	Asian	PCR-RFLP	17	27	23				26	28	8	0.915
Xu	2003	China	Asian	PCR-RFLP	15	33	21	24	21	9	20	25	7	0.853
Wen	2008	China	Asian	PCR-RFLP	22	50	23				27	25	5	0.817
Lin	2009	China	Asian	PCR-RFLP	56	36	47	93	22	24				<0.001
Yue	2006	China	Asian	PCR-RFLP	23	55	34	43	76	21	17	11	2	0.903
Sun	2013	China	Asian	PCR-RFLP	35	53	14				43	53	8	0.128
Yoshioka	2004	Japanese	Asian	PCR-RFLP	21	13	6	71	107	29				0.261
Ukinc	2009	Turkey	Caucasian	PCR	6	16	0	22	8	0				0.399
Sun	2004	China	Asian	PCR	45	53	26	57	23	16	74	34	22	<0.001
Ramanathan	2019	India	Asian	PCR-RFLP	72	71	2				81	19	0	0.294
Nemr(a)	2010	Lebanon	Caucasian	PCR-RFLP	78	104	70	173	100	36				0.001
Nemr(b)	2010	Bahrain	Caucasian	PCR-RFLP	158	58	8	237	86	5				0.371
Dai	2012	China	Asian	PCR-RFLP	22	26	12	29	28	3	31	27	2	0.177
Movva	2011	India	Asian	PCR-RFLP	53	30	0	34	32	0	46	9	0	0.509
Odawara	1999	Japanese	Asian	PCR	52	65	26	38	68	25				0.579
Boger	2007	Germany	Caucasian	PCR	188	219	32	64	69	15				0.566
Eroglu	2007	Turkey	Caucasian	PCR	26	20	1	25	25	6	63	58	7	0.172
El-Baz	2012	Egypt	Caucasian	PCR-RFLP	32	46	24	78	19	3				0.189
Bluthner	1999	Poland	Caucasian	PCR	74	50	23	63	65	18	67	68	15	0.709
Ksiazek	2004	Poland	Caucasian	PCR	77	65	29	82	58	15	71	83	16	0.237
Ma	2019	China	Asian	TaqMan	48	166	107	79	169	86				0.82
Shpichinetsky	2000	Israel	Caucasian	PCR	23	22	10	21	16	6				0.317
Sun	2001	China	Asian	PCR-RFLP	29	55	28	41	35	18	31	16	10	0.008
Shcherbak	1999	Russia	Caucasian	PCR	19	21	11	56	29	5	174	100	23	0.113
Mtiraoui	2007	Tunisia	Caucasian	PCR-RFLP	11	56	26	152	79	36	270	94	36	<0.001
Rahimi	2010	Iran	Caucasian	PCR	60	63	17	45	26	2				0.438

CC = Wild genotype, CT = Heterozygous genotype, DM = Diabetes mellitus, DN = Diabetic nephropathy, HWE = Hardy-Weinberg equilibrium, PCR-RFLP = Polymerase chain reaction-restriction fragment length polymorphism, TT = Homozygous genotype.

2.4. Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was assessed between the controls using the χ^2 test or Fisher exact test; in addition, a *P-value* above.001 demonstrated the fact that the population was in genetic equilibrium. The *ORs* and corresponding *95% CIs* were utilized to quantify the strength of the relationship existing between the MTHFR C677T polymorphism and the susceptibility to DN. Furthermore, the importance of the accumulated OR

Table 2

The results of meta-analysis for different populations in various genotype models (DN vs DM).

Genetic model	Population	Number of studies	Heterogeneity			Test of Association			Test of Egger
			I^2	*P*	Model	Pooled OR	95%CI	*P*-meta	
T vs. C	Overall	24	83.9	<.001	R	1.64	1.34–2.00	<.001	0.576
	Asian	13	73.7	<.001	R	1.54	1.23–1.92	<.001	
	Caucasian	11	89.2	<.001	R	1.76	1.24–2.50	.001	
TC vs. CC	Overall	24	79.9	<.001	R	1.67	1.27–2.21	<.001	0.148
	Asian	13	72.1	<.001	R	1.52	1.07–2.18	.02	
	Caucasian	11	85.6	<.001	R	1.85	1.20–2.86	.005	
TT vs. CC	Overall	22	72.3	<.001	R	2.55	1.82–3.57	<.001	0.628
	Asian	12	51.6	.019	R	2.33	1.66–3.27	<.001	
	Caucasian	10	82	<.001	R	2.75	1.45–5.24	.002	
TT+TC vs.	Overall	24	83.5	<.001	R	1.85	1.40–2.46	<.001	0.235
CC	Asian	13	75.7	<.001	R	1.69	1.19–2.41	.003	
	Caucasian	11	88.6	<.001	R	2.05	1.30–3.23	.002	
TT vs.	Overall	22	52.4	.002	R	1.89	1.50–2.38	<.001	0.454
CC+CT	Asian	12	21.6	.231	R	1.73	1.38–2.17	<.001	
	Caucasian	10	67.3	.001	R	2.07	1.32–3.24	.001	

CC = wild genotype, CI = confidence interval, CT = heterozygous genotype, DM = diabetes mellitus without nephropathy, DN = diabetic nephropathy, F = fixed-effect model, OR = odds ratio, *P*-meta = *P*-value of pooled effect, R = random-effect model, TT = homozygous genotype.

was examined using a Z-test, where $P < .05$ suggested the statistical significance. The between-study heterogeneity was assessed with the Q statistic, Labbe plot, and I^2 statistic.[12,13] The fixed-effect framework (Mantel-Haenszel method) was conducted at $P_h > 0.1$ or at $I^2 < 50\%$[14]; otherwise, the random-effect framework (DerSimonian-Laird method) was applied.[15] Moreover, subgroup analysis was conducted on the basis of ethnicity. The sensitivity analysis was performed through omitting each study individually to evaluate the robustness of the findings. Begg funnel plot as well as Egger test were undertaken to evaluate latent publication bias.[16,17] All statistical analyses were carried out using STATA software version 15.0 for Windows.

3. Results

3.1. Characteristics of the studies

Figure 1 sheds light on the literature search process. An aggregate of 165 pertinent research works was formed from the preliminary search of databases. Four replicated publications were removed in the initial screening. After screening titles and abstracts, 137 unrelated articles were excluded. Moreover, the remaining papers were subjected to a full-text review by 2 independent authors. Eventually, 28 qualifying studies were included in the current study.[6,18–44] Among 8787 participants, 4154 were Asian while 4633 participants were Caucasian. Among all 28 research populations, the allocations of the MTHFR C677T polymorphism in the controls were in alignment with HWE, except for three research studies.[27,31,40]Table 1 summarizes the attributes of the registered research works as well as the HWE examination findings.

3.2. The MTHFR C677T polymorphism and DN (DN vs diabetes mellitus [DM]))

The heterogeneity was assessed with the Q statistic, Labbe plot, and I^2 statistic in 5 genetic frameworks. As presented in Table 2, significant heterogeneity was detected in 5 genetic models; accordingly, the random-effect framework was adopted in this analysis. The Labbe plots for the MTHFR C677T polymorphism in the allelic and recessive models are presented in Figure 6. As the findings suggested, there was a significant relationship between the polymorphism of C677T and an augmented susceptibility to DN compared with that to DM in allelic ($OR = 1.64$, 95% $CI = 1.34–2.00$, $P < .001$), dominant ($OR = 1.85$, 95% $CI = 1.40–2.46$, $P < .001$), codominant (heterozygote: $OR = 1.67$, 95% $CI = 1.27–2.21$, $P < .001$; homozygote: $OR = 2.55$, 95% $CI = 1.82–3.57$, $P < .001$), and recessive ($OR = 1.89$, 95% $CI = 1.50–2.38$, $P < .001$) frameworks in the populations in general (Fig. 2). Owing to the substantial between-study heterogeneity in the

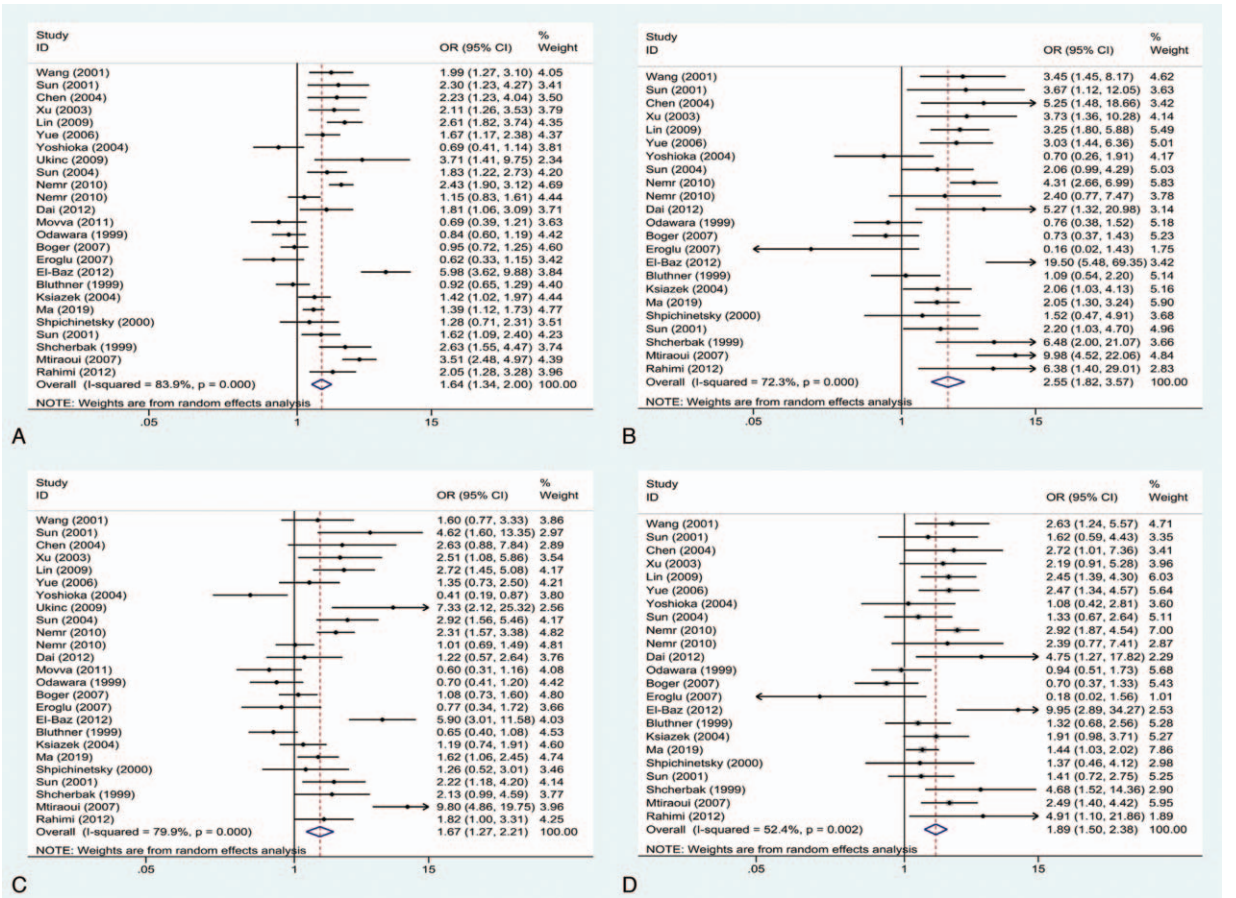

Figure 2. Forest plots for the association between MTHFR C677T polymorphism and diabetic nephropathy susceptibility (compared diabetic nephropathy group with diabetes mellitus group). (A) allelic model; (B) homozygote model; (C) heterozygote model; (D) recessive model.

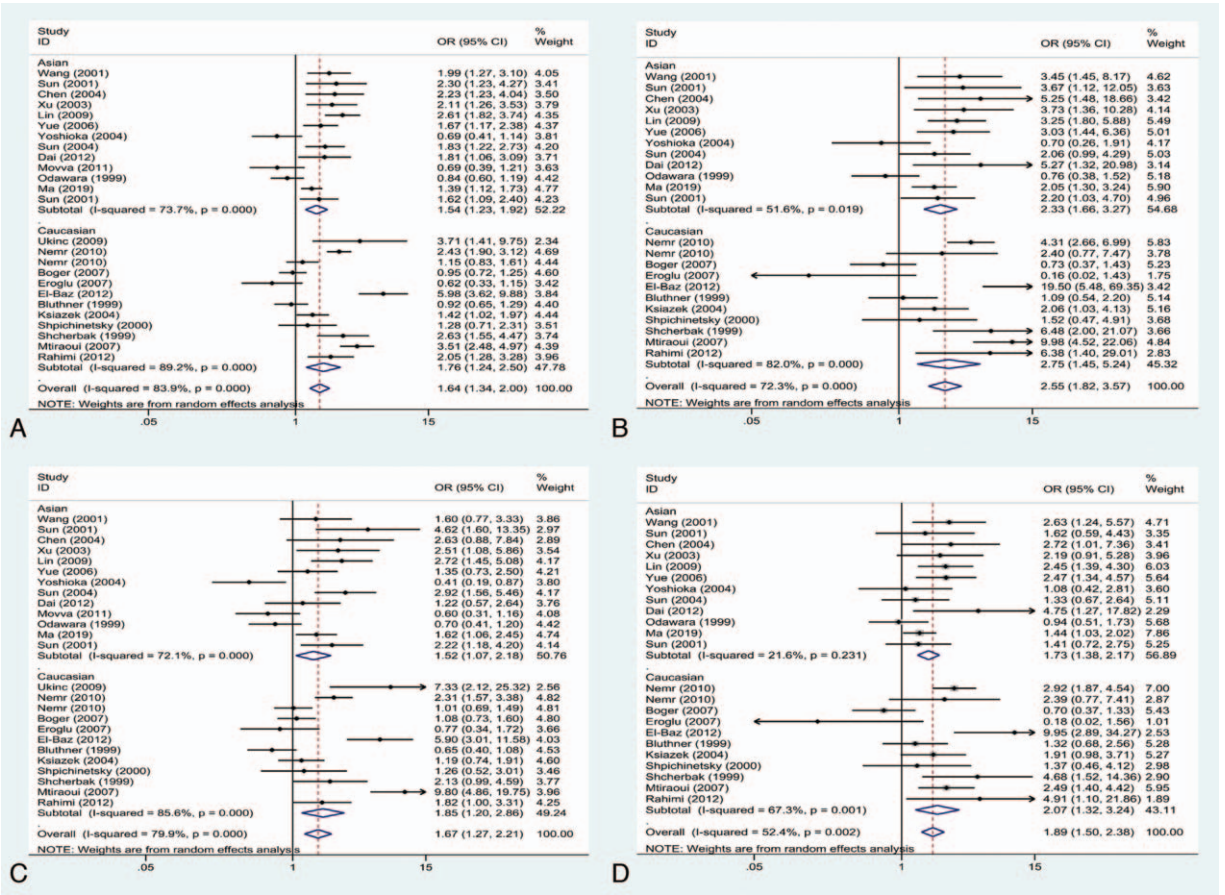

Figure 3. Forest plots for the association between MTHFR C677T polymorphism and diabetic nephropathy susceptibility stratified analyses according to ethnicity (compared diabetic nephropathy group with diabetes mellitus group). (A) allelic model; (B) homozygote model; (C) heterozygote model; (D) recessive model.

earlier comparisons, we carried out subgroup analysis based on ethnicity, and no substantial changes were observed in the risk estimations in all genetic comparison frameworks. The stratified analysis based on ethnicity revealed significantly enhanced susceptibility to DN in Caucasian and Asian populations, as shown in Figure 3. In addition, three of the research works[27,31,40] had genotype distributions of the C677T polymorphism in DM controls that deviated from HWE; however, the accumulated ORs still reached significance in all genetic comparison models after excluding these three research studies. The key results are shown in Table 2.

3.3. The MTHFR C677T polymorphism and DN (DN vs healthy control)

The influence of the MTHFR C677T polymorphism on DN susceptibility was evaluated in 18 research works. Significant heterogeneity was observed in the genetic comparison models except for the recessive genetic model; accordingly, the random-effect model was adopted to evaluate the correlation existing between the MTHFR C677T polymorphism and DN susceptibility. Furthermore, the Labbe plots for the MTHFR C677T polymorphism in the allelic and recessive models are shown in Figure 6. The overall analysis shed light on the fact that the MTHFR C677T polymorphism had a significant correlation with an augmented susceptibility to DN in all five genetic comparison frameworks (allelic model: $OR = 2.06$, $95\%\ CI = 1.58–2.67$, $P < .001$; dominant model: $OR = 2.52$, $95\%\ CI = 1.73–3.69$, $P < .001$; codominant model: $OR = 3.78$, $95\%\ CI = 2.50–5.70$, $P < .001$; recessive model: $OR = 2.41$, $95\%\ CI = 1.96–2.97$, $P < .001$), as shown in Figure 4. Owing to the substantial between-study heterogeneity determined in the earlier comparisons, subgroup analysis was carried out on the basis of ethnicity, and no substantial change was observed in the risk estimations in all genetic comparison frameworks. The subgroup analysis based on ethnicity showed a substantial increase in susceptibility to DN among Asian populations in the 5 genetic comparison frameworks; however, a significant association in Caucasian populations was found only in the recessive genetic model ($OR = 2.34$, $95\%\ CI = 1.68–3.24$, $P < .001$), as presented in Figure 5. Moreover, in 2 studies,[31,40] the genotype distributions of the C677T polymorphism in DM controls deviated from HWE, and the accumulated ORs still reached significance in all genetic comparison models after excluding these 3 studies.

3.4. Sensitivity analysis and publication bias

To assess whether a sole research work could impact the final ORs, each separate research work was eliminated once, after which the data was repooled. The analysis findings illustrated that the accumulated ORs were not affected by the deletion of individual studies, as shown in Figure 7. Begg funnel plot, together with Egger test, was employed to evaluate the publication partiality. All plots were observed as having

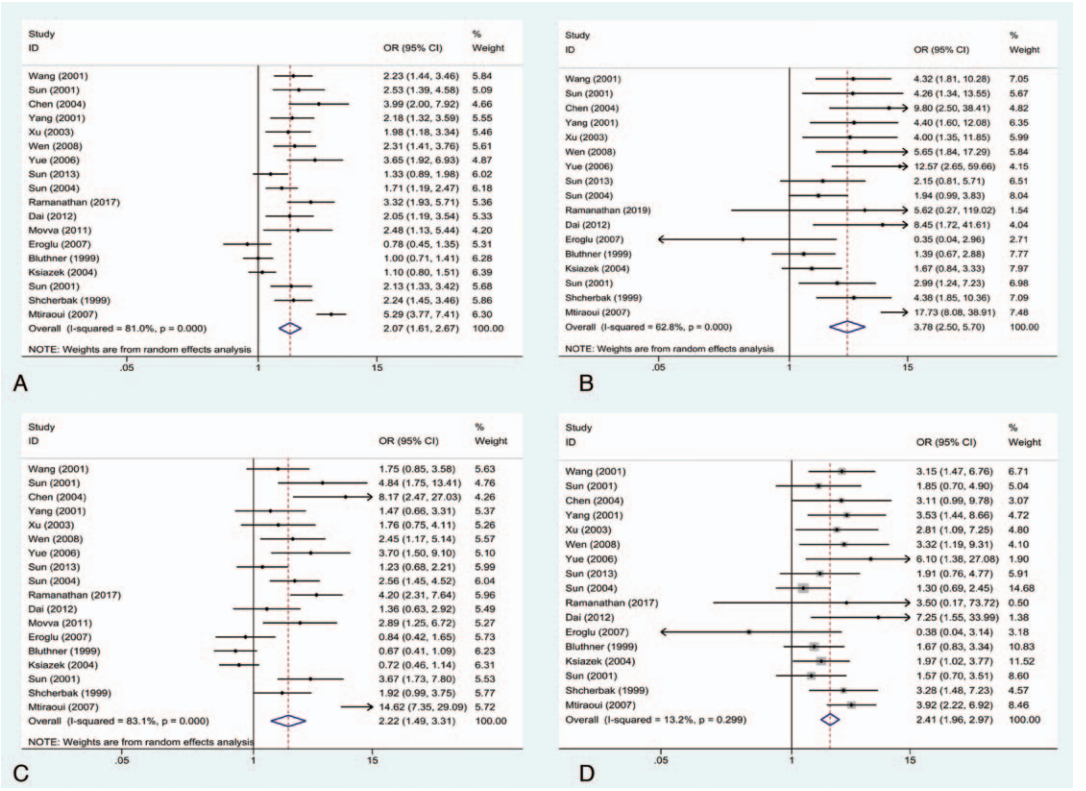

Figure 4. Forest plots for the association between MTHFR C677T polymorphism and diabetic nephropathy susceptibility (compared diabetic nephropathy group with healthy control group). (A) allelic model; (B) homozygote model; (C) heterozygote model; (D) recessive model.

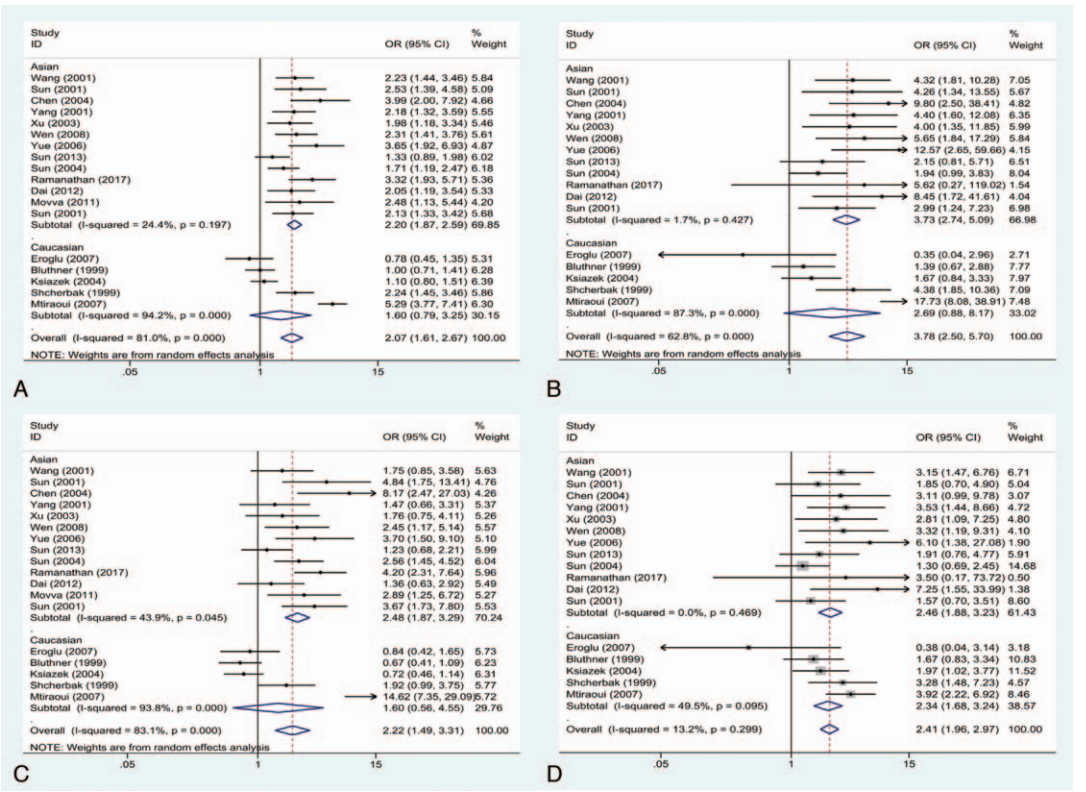

Figure 5. Forest plots for the association between MTHFR C677T polymorphism and diabetic nephropathy susceptibility stratified analyses according to ethnicity (compared diabetic nephropathy group with healthy control group). (A) allelic model; (B) homozygote model; (C) heterozygote model; (D) recessive model.

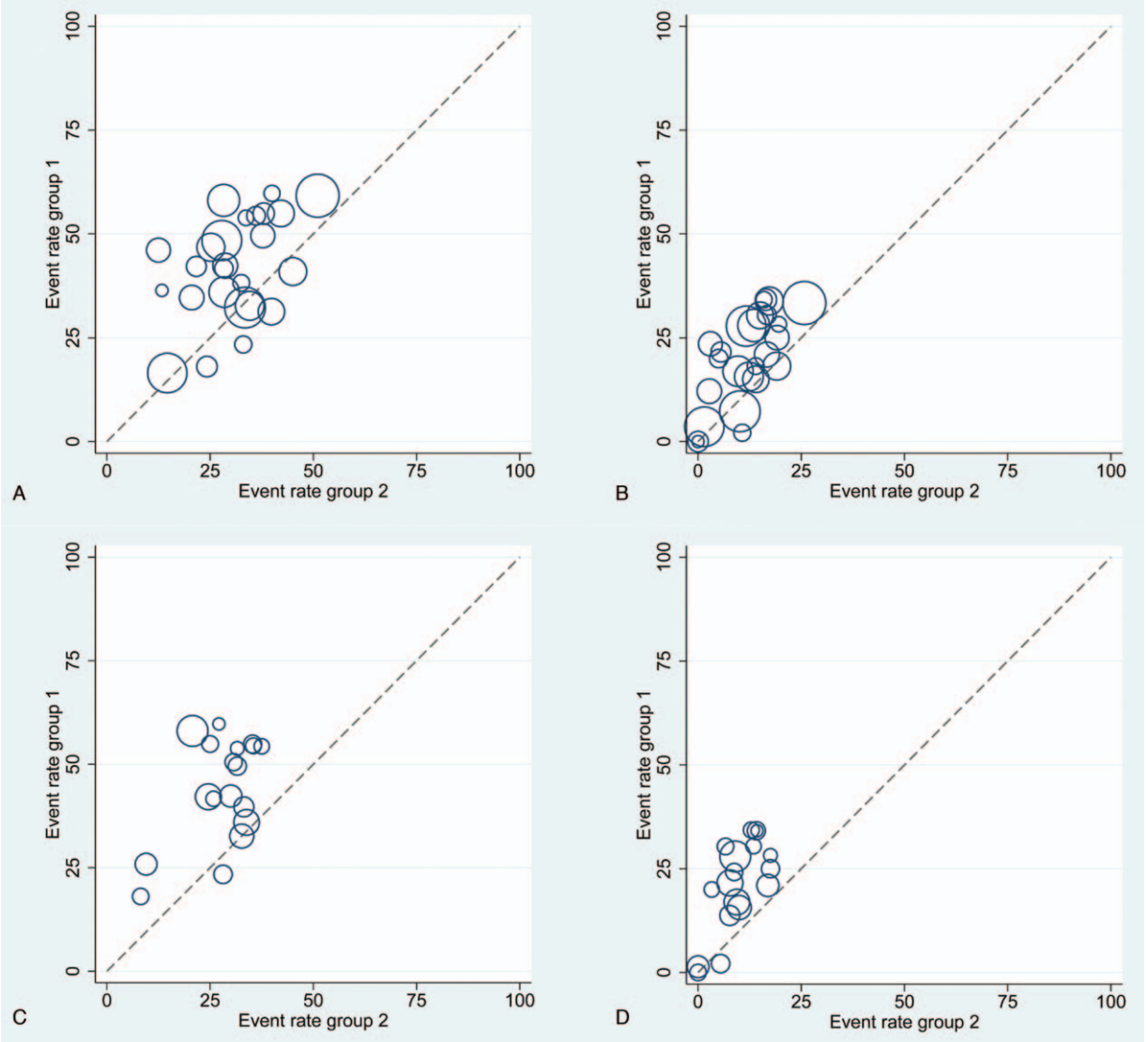

Figure 6. Labbe plots of the included studies focusing on the association between MTHFR C677T polymorphism and diabetic nephropathy susceptibility. Compared DN group with diabetes mellitus group in allelic model (A), and recessive model (B); compared DN group with healthy control group in allelic model (C), and recessive model (D). DN = diabetic nephropathy.

approximate symmetry, which suggested that no evident publication bias was present. Moreover, the main results are presented in Figure 8 and Tables 2 and 3.

4. Discussion

Diabetes and its related complications represent a substantial health and economic load; in addition, given the rising epidemics of obesity as well as diabetes among children and young people, the occurrence of diabetes is anticipated to continue growing. Furthermore, the pathophysiology of DN remains ambiguous, thus requiring further investigation. There appears to be an inherited predisposition for DN; in addition, there are some candidate genes that have been reproducibly connected to DN.[5,7] Gene studies are likely to offer worthwhile information about the pathobiology of DN as well as the latest targets for its therapy.[1]

The pathogenesis of DN is multifactorial, and the high level of plasma homocysteine is considered a key risk factor for the development of DN.[45] Moreover, homocysteine is considered as an intermediary sulfur compound that contains the product of methionine metabolism, whereas its levels are influenced by the levels of vitamin B12 and folic acid.[46] MTHFR constitutes a major regulatory enzyme in homocysteine and folate metabolism.[7] The polymorphism of MTHFR C677T is likely to exert an effect on the step in homocysteine metabolism in which it is involved.[7,46] The homozygous variants of MTHFR C677T have higher levels of homocysteine, whereas the heterozygous variants have moderately augmented levels of homocysteine in comparison with the homozygous wild-type genotype.[10,47] Accordingly, there exists biological evidence for the correlation of the polymorphism of MTHFR C677T with DN susceptibility.

In 1998, Neugebauer, together with colleagues, first proposed a correlation between the polymorphism of MTHFR C677T and

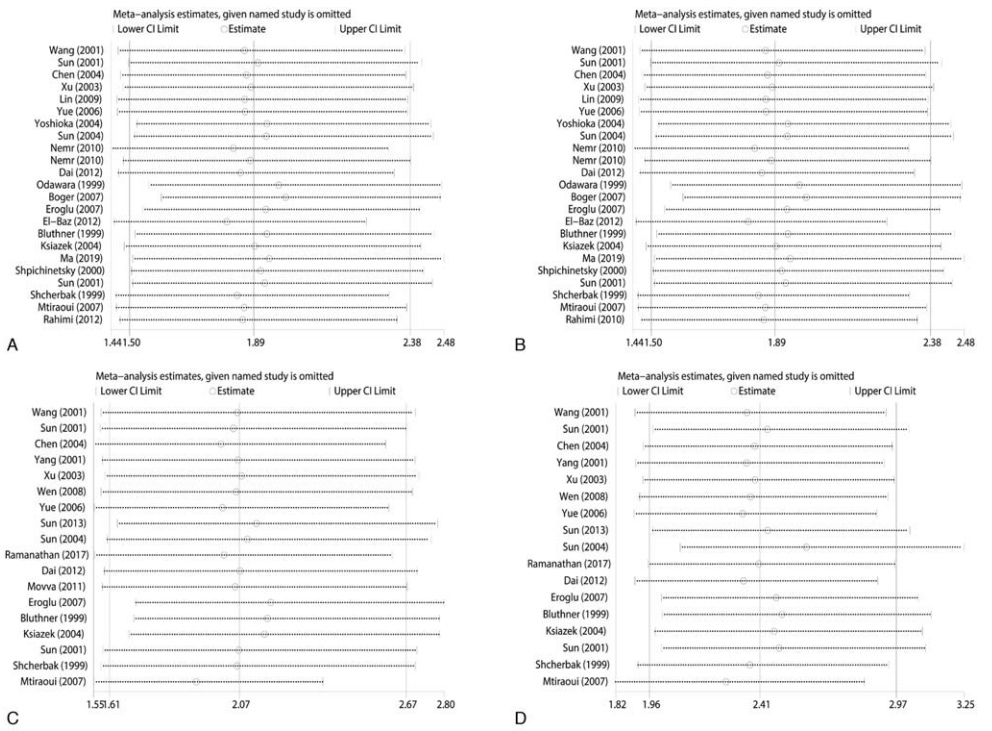

Figure 7. Sensitivity analysis for the included studies focusing on the relationship of the polymorphism of MTHFR C677T on the susceptibility to diabetic nephropathy. Compared DN group with diabetes mellitus group in allelic model (A), and recessive model (B); compared DN group with healthy control group in allelic model (C), and recessive model (D). DN = diabetic nephropathy.

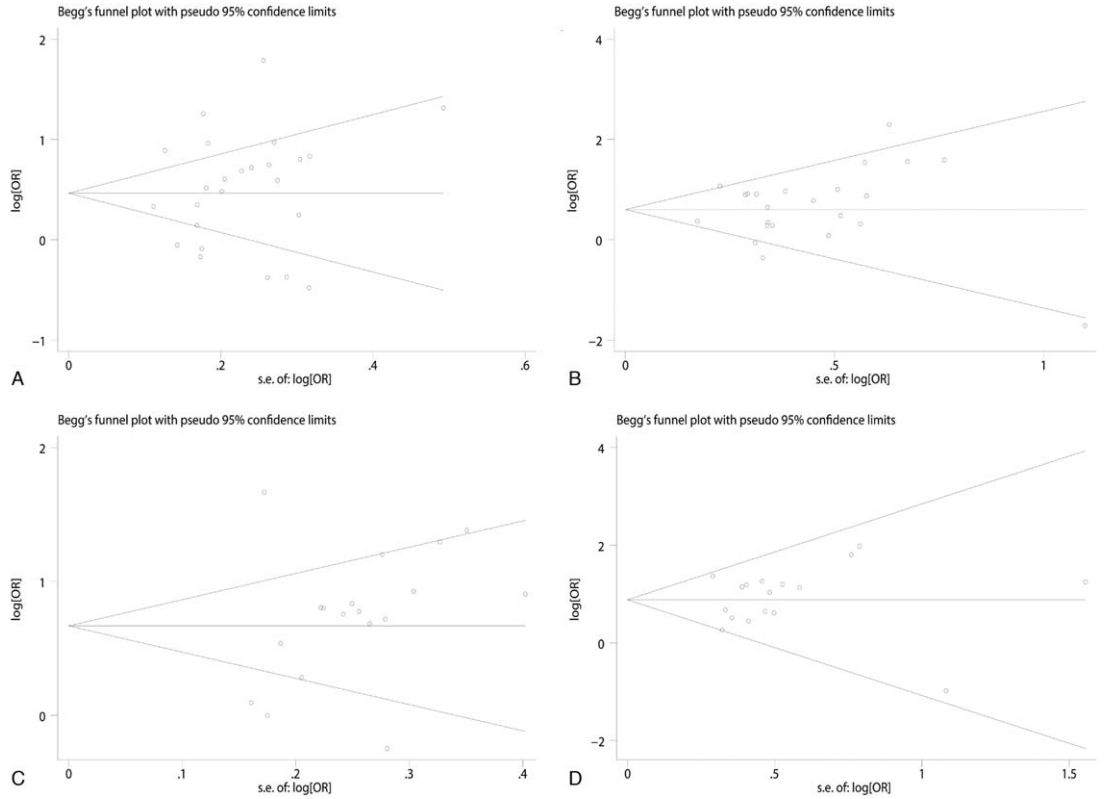

Figure 8. Begg funnel plots of publication bias for the relationship of the polymorphism of MTHFR C677T on the susceptibility to diabetic nephropathy. Compared DN group with diabetes mellitus group in allelic model (A), and recessive model (B); compared DN group with healthy control group in allelic model (C), and recessive model (D). DN = diabetic nephropathy.

Table 3

The results of meta-analysis for different populations in various genotype models (DN vs healthy control).

Genetic model	Population	Number of studies	Heterogeneity			Test of association			Test of Egger
			I^2	*P*	Model	Pooled OR	95%CI	*P*-meta	
T vs C	Overall	17	82.1	<.001	R	2.06	1.58–2.67	<.001	0.26
	Asian	12	30.2	.151	R	2.2	1.85–2.62	<.001	
	Caucasian	5	94.2	<.001	R	1.6	0.79–3.25	.192	
TC vs CC	Overall	18	83.1	<.001	R	2.22	1.49–3.31	<.001	0.016
	Asian	13	43.9	.045	R	2.48	1.87–3.29	.001	
	Caucasian	5	93.8	<.001	R	1.6	0.56–4.55	.379	
TT vs CC	Overall	17	62.8	<.001	R	3.78	2.50–5.70	<.001	0.354
	Asian	12	1.7	.427	R	3.73	2.74–5.09	<.001	
	Caucasian	5	87.3	<.001	R	2.69	0.88–8.17	.081	
TT+TC vs	Overall	18	83.4	<.001	R	2.52	1.73–3.69	<.001	0.007
CC	Asian	13	35.5	.099	R	2.8	2.18–3.58	<.001	
	Caucasian	5	94.2	<.001	R	1.8	0.65–4.96	.257	
TT vs	Overall	17	13.2	.299	F	2.41	1.96–2.97	<.001	0.668
CC+CT	Asian	12	0	.469	F	2.46	1.88–3.23	<.001	
	Caucasian	5	49.5	.095	F	2.34	1.68–3.24	<.001	

CC = wild genotype, CI = confidence interval, CT = heterozygous genotype, DN = diabetic nephropathy, F = fixed-effect model, OR = odds ratio, *P*-meta = *P*-value of pooled effect, R = random-effect model, TT = homozygous genotype.

the susceptibility to DN, and the findings illustrated that this polymorphism likely contributes to the development of DN.[11] Consequently, Chang et al carried out a meta-analysis for this association, which indicated that the polymorphism of MTHFR C677T might influence the susceptibility to DN in the Chinese population.[48] In 2016, Xiong and colleagues examined only Chinese studies examining the correlation of the polymorphism of MTHFR C677T with the susceptibility to DN.[49] To attain a more accurate approximation of this correlation, a meta-analysis was carried out in the present study. To our knowledge, our study constitutes the most detailed research addressing the association between the MTHFR C677T polymorphism and DN susceptibility. Moreover, 28 studies involving 8787 participants were included in this analysis. Overall, we elucidated that the polymorphism of MTHFR C677T substantially augmented the susceptibility to DN in not only Asian, but also Caucasian populations.

This study has some limitations. Firstly, the sample size of some studies was limited, which might give rise to bias in the results when assessing the correlation of the polymorphism of MTHFR C677T with the susceptibility to DN. Secondly, the present study was statistically heterogenic, although this is highly frequent in meta-analyses of genetic correlations. Hence, we implemented subgroup analysis to identify all determinants that contributed to heterogeneity. Thirdly, other determinants that are likely to affect the correlation of the MTHFR C677T polymorphism with the susceptibility to DN, such as sex, environment, and lifestyle, could not be analyzed due to a lack of genuine data. Ultimately, only published studies were included in this analysis. Moreover, unpublished works and further studies may be capable of altering our findings. Based on the abovementioned reasons, the pooled estimates of our meta-analysis require careful interpretation.

5. Conclusions

To summarize, the present study suggests that the MTHFR C677T polymorphism is likely to be related to an augmented susceptibility to DN in not only Asian but also Caucasian populations. Nonetheless, prospective studies with effective designs and extensive sample sizes might be beneficial for the validation of this association in various ethnicities.

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