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Original Article

MTHFR gene C677T polymorphism and type 2 diabetic nephropathy in Asian populations: a meta-analysis

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Abstract: Background: Many studies have suggested a correlation between the C677T mutation in the methylenetetrahydrofolate reductase (*MTHFR*) gene and diabetic nephropathy (DN), but their results are inconclusive. Methods: To confirm this correlation, we performed a meta-analysis of 15 studies. The dichotomous data are presented as odds ratios (*OR*) with 95% confidence intervals (*CI*). Results: The results of this study suggested that the *MTHFR* 677 T allele was more likely to increase the risk of DN in Asian (*OR* = 1.466, 95% *CI* = 1.143-1.880, *P* = 0.003), West Asian (*OR* = 1.750, 95% *CI* = 1.150-2.664, *P* = 0.009), and Chinese populations (*OR* = 2.162, 95% *CI* = 1.719-2.719, *P* = 0.001), but not in East Asian or Japanese populations. The carriers of the *MTHFR* 677 T allele were associated with progression of DN in the "5-10 year duration" group, but not in the "> 10 year duration" group (*OR* = 2.187, 95% *CI* = 1.787-2.677, *P* = 0.001). Conclusion: Development of DN is associated with *MTHFR* C677T polymorphisms in Asian populations, especially in early type 2 diabetes.

Keywords: Diabetic nephropathy, *MTHFR* polymorphisms, type 2 diabetes

Introduction

Diabetic nephropathy (DN) is the leading cause of chronic renal disease and a major cause of cardiovascular mortality. Diabetic nephropathy is associated with cardiovascular disease and increases mortality of diabetic patients [1]. Several factors are involved in the pathophysiology of DN, and genetic susceptibility to type 1 and type 2 diabetes is of great importance [2]. A familial study has provided compelling evidence that genetic factors contribute to DN susceptibility in type 2 diabetes as have studies aimed at identifying the causal genes responsible for its development. The methylenetetrahydrofolate reductase (MTHFR) is an enzyme that catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. Homozygosity for the C to T substitution at nucleotide 677 of the *MTHFR* gene leads to a 50% reduction in enzyme activity and is the most common inherited cause of moderate hyperhomocysteinemia. This polymorphism is located in the catalytic domain of the enzyme and results in the production of a thermolabile protein. Many studies have investigated *MTHFR* gene polymorphism effects on susceptibility to type 2 DN, but the results are inconclusive. We have summarized the findings of those individual studies in **Table 1**.

Meta-analysis is a powerful method for quantitatively summarizing results from different studies. One of its advantages is to increase the sample size, which may reduce the probability that random error will result in a false-positive or false-negative association. Therefore, we performed a meta-analysis to quantitatively assess the association of *MTHFR* gene polymorphisms with DN.

Materials and methods

Literature search strategy

The Medline, PubMed, Embase, and Web of Science were searched (the last search was updated on October 19, 2014 by using the search terms: "Diabetic Nephropathy" or "DN",

Table 1. Findings of the studies included in this meta-analysis

Studies	Year	Country	Number		Genotype Assay	Source Of Control	HWE Result	Quality Assessment	Conclusion
			DNs	DMs					
Ravindra Kumar [4]	2013	India	407	185	PCR-RFLP	Hospital-based	0.58	8.5	The MTHFR C677T polymorphism plays a significant role in predisposition of renal insufficiency in diabetic patients
Mehrali Rahimi [5]	2010	Iran	140	72	PCR-RFLP	Hospital-based	0.75	6	Both MTHFR 677T and 1298C alleles increased the susceptibility to the onset and progression of DN in Iranians with T2DM
Rita Nemr [6]	2010	Lebanon	252	309	PCR-RFLP	Hospital-based	0.01	9	The contribution of C677T single nucleotide polymorphism to increased risk of DN (presumably by increasing homocysteine concentrations) must be evaluated in the context of the ethnic background
	2010	Bahrain	224	328	PCR-RFLP	Hospital-based	0.37	9	
Kubilay Ukinc [7]	2009	Turkey	22	30	PCR-RFLP	Hospital-based	0.80	6	There was a significantcorrelation of plasma homocysteine level with microalbuminuria ($r = 0.54$; $P = 0.031$) in the patients with diabetic nephropathy who had C677T polymorphism
K. Yoshioka [8]	2004	Japan	135	207	PCR-RFLP	Hospital-based	0.26	7	The MTHFR gene mutation is not related both to the development of diabetic nephropathy and diabetic retinopathy in Japanese Type 2 diabetic patients
Jiazhong Sun [9]	2004	China	124	124	PCR-RFLP	Hospital-based	0.01	7	MTHFR C677T gene polymorphism associated with a predisposition to increased plasma homocysteine levels may represent a genetic risk factor for diabetic nephropathy in Chinese type 2 diabetic patients
G Hasegawa [10]	2002	Japan	135	207	PCR-RFLP	Hospital-based	0.58	7	The MTHFR 677T allele, together with renal dysfunction due to diabetic nephropathy, could be a strong risk factor atherosclerotic disease
Longqing Wang [11]	2001	China	82	79	PCR-RFLP	Hospital-based	0.44	6.5	The MTHFR 677T allele may be contributed to the risk of diabetic nephropathy
Vlad Shpichinetsky [12]	2000	Israel	55	43	PCR-RFLP	Hospital-based	0.17	6	Folate supplementation in diabetic patients with the C677T mutation and low-normal serum folate may prevent the onset or retard the progression of DN
Hiroki Fujita [13]	1999	Japan	105	68	PCR-RFLP	Hospital-based	0.28	6	The MTHFR gene polymorphism is not associated with the development of diabetic nephropathy in Japanese type II diabetic patients
M.Odawara [14]	1999	Japan	143	131	PCR-RFLP	Hospital-based	0.58	7.5	Although previous observations suggest a role for the C677T mutation of the MTHFR gene in the development of diabetic nephropathy, it seems to be premature to conclude that this mutation is associated with diabetic nephropathy in Japanese people
Sabine Neugebauer [15]	1998	Japan	67	63	PCR-RFLP	Hospital-based	0.03	6.5	The MTHFR gene polymorphism is associated with diabetic nephropathy and may contribute to the progression of nephropathy by aggravating renal vascular injury
Makiko Maeda [16]	2008	Japan	72	87	PCR-RFLP	Hospital-based	0.05	6	Our results are highly suggestive of an important role for MTHFR genotype in susceptibility to retinopathy under hyperglycemia, but not to nephropathy
L Lin [17]	2009	china	139	139	PCR-RFLP	Hospital-based	0.01	7	The MTHFR 677T allele may be contributed to he risk of diabetic nephropathy

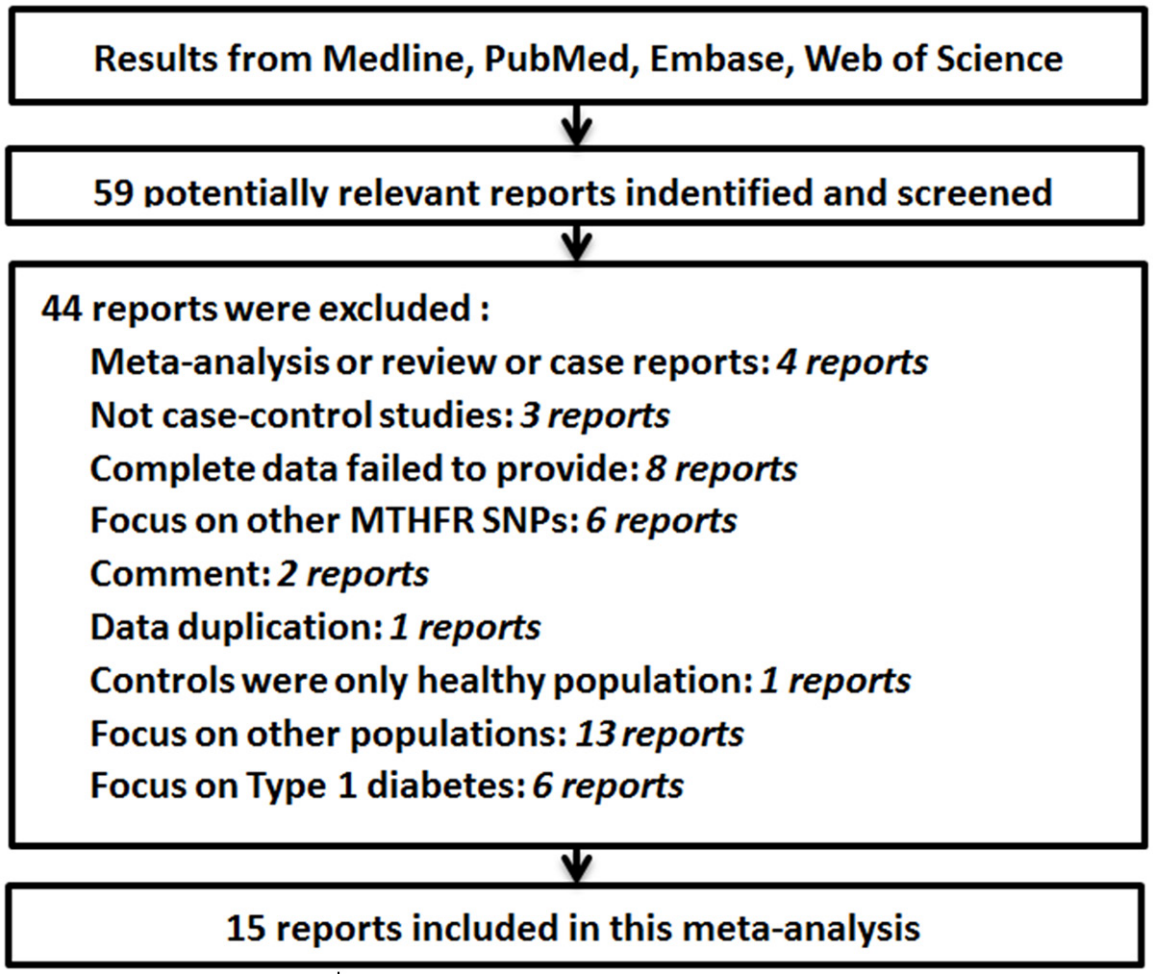

Figure 1. A flow diagram of the study selection process.

"polymorphism", "MTHFR" or "Methylenetetrahydrofolate reductase" All searched studies were retrieved and their bibliographies were checked for other relevant publications. Review articles and bibliographies of other relevant identified studies were hand-searched in addition to eligible studies. Only published studies with full-text articles were included. When the same patient population was included in several publications, only the most complete study or the study with the largest sample size was used in this meta-analysis. A flow diagram of the study selection process is shown in **Figure 1**.

Inclusion and exclusion criteria

The inclusion and exclusion criteria were determined by this research team discussion. The inclusion criteria were: (1) the study aimed to examine the association between *MTHFR* polymorphisms and susceptibility to DN; (2) the design of the study was a case-control study; (3) the study used diabetic patients without nephropathy or healthy subjects as controls; (4) the study provided the number of DN cases or controls and the frequency of *MTHFR* genotypes. The exclusion criteria were: (1) the study did not fit the diagnostic criteria; (2) the study was conducted on animals; (3) the study was not a case-control study; (4) the reported data was not adaptable for our pooled study; (5) the study focused on type 1 diabetic subjects.

Data extraction

All of the data were extracted independently by 2 reviewers (Hai-yan Chen and Fang Wei) according to the pre-specified selection criteria. Disagreement was resolved by discussion. The following data were extracted: control type, diabetic duration, study design, first author's

Table 2. Summary about meta-analysis on MTHFR C677T polymorphisms in Asian type 2 diabetes patients (with nephropathy vs.without nephropathy) P_B: *P* for Begg' test; P_E: *P* for Egger' test.

Comparisons	stratification	Subgroups	n	OR (95% CI)			Homogeneity			Publication Bias	
				OR	*CI*	*P value*	*Q*	*Ph*	I^2 (%)	P_B	P_E
Allele model (T vs. C)	over-all		16	1.466	1.143-1.880	0.003	79.70	0.001	82.4	0.805	0.918
	Region	East Asia	8	1.289	0.934-1.778	0.123	18.44	0.001	78.3	0.231	0.182
		West Asia	5	1.750	1.150-2.664	0.009	45.95	0.001	82.6	0.532	0.371
	Nation	Japanese	6	0.975	0.768-1.237	0.835	10.24	0.069	51.2	0.343	0.433
		Chinese	3	2.162	1.719-2.719	0.001	1.86	0.394	0.0	0.783	0.832
	Diabetes duration (years)	> 10	9	1.141	0.830-1.570	0.417	50.46	0.001	84.1	1.000	0.276
		5 < and < 10	5	2.187	1.787-2.677	0.001	2.96	0.564	0.0	0.49	0.558
Recessive (TT vs. TC+CC)	over-all		16	1.630	1.229-2.162	0.001	24.08	0.045	41.9	0.621	0.733
	Region	East Asia	9	1.397	1.028-1.898	0.033	12.54	0.129	36.2	1.000	0.829
		West Asia	5	2.555	1.749-3.732	0.001	4.01	0.404	1.30	0.763	0.652
	Nation	Japanese	6	1.078	0.789-1.474	0.636	3.33	0.650	0.0	0.835	0.813
		Chinese	3	2.069	1.373- .117	0.001	2.34	0.311	14.4	0.721	0.975
	Diabetes duration (years)	> 10	9	1.378	0.941-2.017	0.099	17.33	0.027	53.8	0.49	0.729
		5 < and < 10	5	2.139	1.481-3.089	0.001	3.65	0.456	0.0	0.297	0.269
Dominant (TT+TC vs. CC)	over-all		16	1.552	1.108- 2.173	0.011	80.43	0.001	82.6	0.921	0.955
	Region	East Asia	9	1.300	0.807- 2.094	0.280	49.16	0.001	80.7	0.532	0.422
		West Asia	5	1.989	1.133-3.494	0.017	20.78	0.001	83.7	0.543	0.231
	Nation	Japanese	6	0.910	0.586-1.413	0.674	16.75	0.005	70.1	0.636	0.873
		Chinese	3	2.621	1.903-3.621	0.001	0.69	0.709	0.0	0.941	0.763
	Diabetes duration (years)	> 10	9	1.097	0.709-1.697	0.679	49.55	0.001	83.9	0.624	0.437
		5 < and < 10	5	2.660	2.022-3.499	0.001	3.63	0.458	0.0	0.677	0.276

name, publication year, and number of DN and diabetes mellitus (DM) subjects. The quality scoring system was first reported by Thakkinstian [3]. Total scores ranged from 0 (lowest) to 10 (highest). Articles with scores equal to or higher than 7 were considered "high-quality" studies, whereas those with scores less than 7 were considered "low-quality" studies. The predefined criteria were shown in Table S1.

Statistical analysis

Allele frequencies at the *MTHFR* single-nucleotide polymorphisms (SNPs) from the studies were determined by the allele counting method. Statistical analysis was conducted using Stata 11.0 (StataCorp, College Station, TX) and a *P* value < 0.05 was considered statistically significant. Dichotomous data are presented as the odds ratio (*OR*) with a 95% confidence interval (*CI*). Statistical heterogeneity was measured using the *Q*-statistic (*P* < 0.10 was considered to be representative of statistically significant heterogeneity). We also quantified the effect of heterogeneity using the I^2 statistic, which measures the degree of inconsistency in the studies by calculating what percentage of the total variation across studies is due to heterogeneity rather than by chance. A fixed-effects model was used when there was no heterogeneity of the results of the trials; otherwise, the random effects model was used. For dichotomous outcomes, patients with incomplete or missing data and small-sample studies were included in the sensitivity analyses by counting them as treatment failures. To establish the effect of clinical heterogeneity between studies on the conclusions of meta-analyses, subgroup analysis was conducted on the basis of race. Several methods were used to assess the potential for publication bias. Visual inspection of asymmetry in funnel plots was conducted. Begg's rank correlation method and Egger's weighted regression method were also used to statistically assess the publication bias (*P* < 0.05 was considered representative of a statistically significant publication bias).

Characteristics of studies

This meta-analysis included 15 relevant reports of *MTHFR* C677T mutation, with 2102 DN subjects and 2072 type 2 DM subjects. The characteristics and results of each study are presented in **Table 1**.

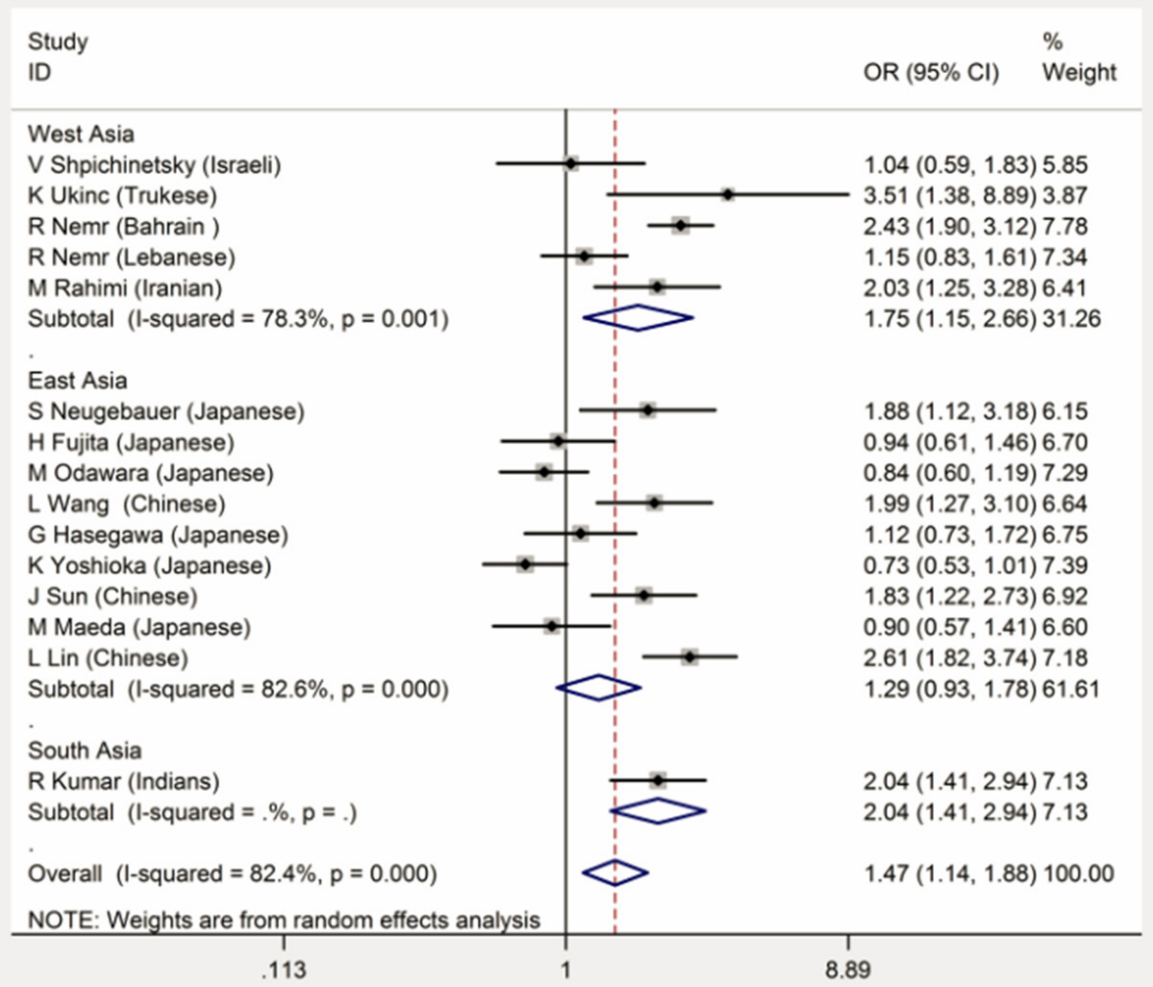

Figure 2. Forest plot of the MTHFR C677T polymorphism and DN stratified by region (T allele vs C allele).

Quantitative data synthesis

The aim of this study was to use the meta-analysis method to quantitatively summarize the results from the selected individual studies. In comparing DN cases versus diabetic patients without nephropathy, our aim was to evaluate the relationship between *MTHFR* C677T polymorphisms and the progress of diabetic patients. The carriers of the *MTHFR* 677 T allele were more likely to have DN than the carriers of the C allele in the overall group and the West Asian subgroup but not in the East Asian subgroup (allele model: overall pooled *OR* = 1.466, 95% *CI* = 1.143-1.880, *P* = 0.003; West Asian group *OR* = 1.750, 95% *CI* = 1.150-2.664, *P* = 0.009; Recessive model: overall pooled *OR* = 1.630, 95% *CI* = 1.229-2.162, *P* = 0.001; West Asian group *OR* = 2.555, 95% *CI* = 1.749-3.732, *P* = 0.001; East Asian group *OR* = 1.397, 95% *CI* = 1.028-1.898, *P* = 0.033; as shown in **Table 2** and **Figure 2**).

To understand the different relationship between the *MTHFR* C677T mutation and the development of diabetes in Chinese and Japanese populations, we performed subgroup analysis by nation. As shown in **Table 2**, the carriers of the *MTHFR* 677 T allele were associated with DN in the Chinese, but not in the Japanese population (allele model: *OR* = 2.162, 95% *CI* = 1.719-2.719, *P* = 0.001; recessive model: *OR* = 2.069, 95% *CI* = 1.373-0.117, *P* = 0.001; dominant model: *OR* = 2.621, 95% *CI* = 1.903-3.621, *P* = 0.001; as shown in **Table 2** and **Figure 3**).

To understand the influence of diabetes duration on the development of diabetes, we divided the included studies into 2 groups based on the average duration of diabetes: the "> 10 year duration" group and the "5-10 year duration" group. As **Table 2** and **Figure 3** show, the carriers of the *MTHFR* 677 T allele were associated with progression of DN in the "5-10 year

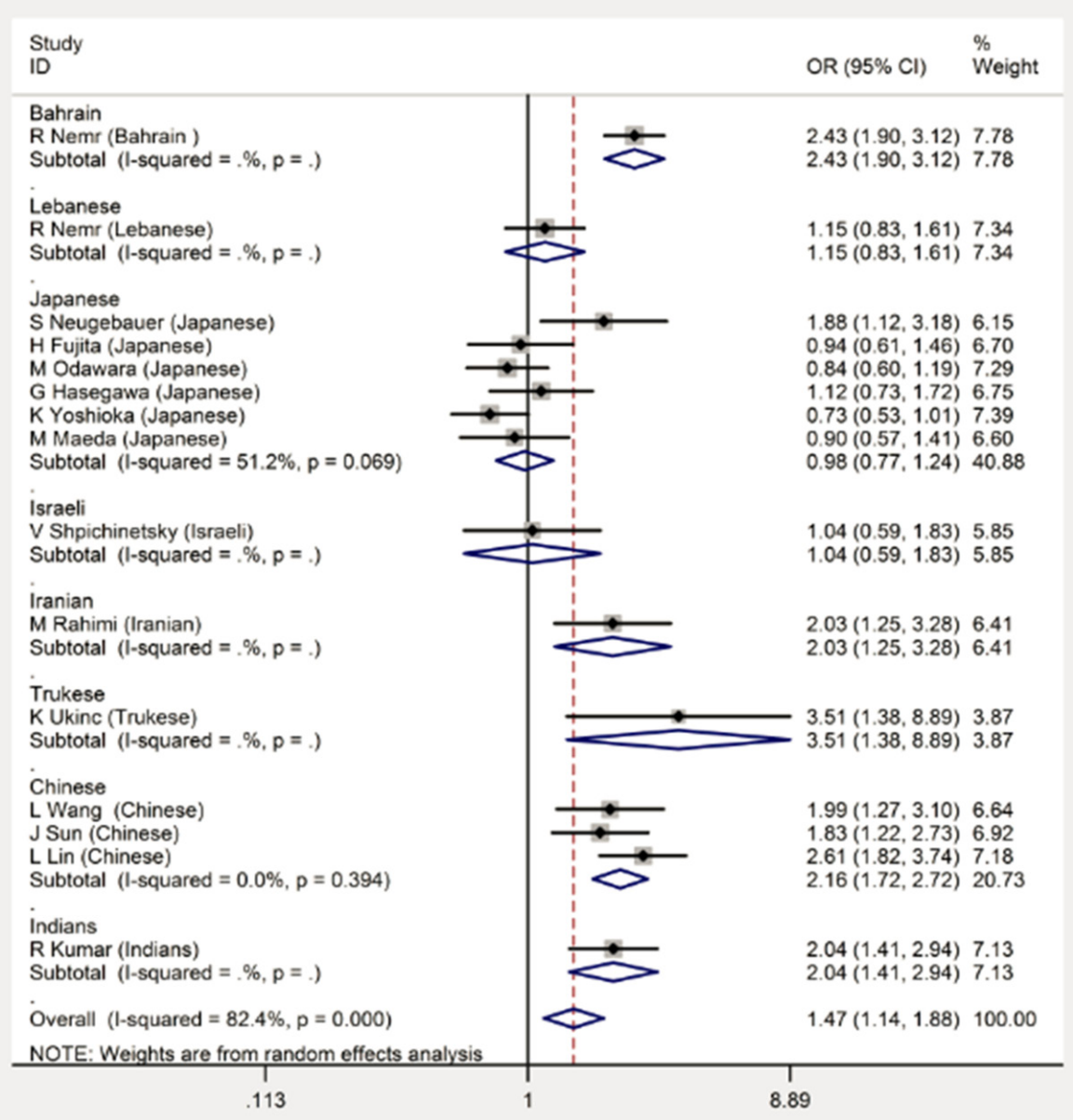

Figure 3. Forest plot of the MTHFR C677T polymorphism and DN stratified by nation (T allele vs C allele).

duration", but not in the "> 10 year duration" group ("5-10 year duration" allele model: *OR* = 2.187, 95% *CI* = 1.787-2.677, *P* = 0.001; recessive model: *OR* = 2.139, 95% *CI* = 1.481-3.089, *P* = 0.001; dominant model: *OR* = 2.660, 95% *CI* = 2.022-3.499, *P* = 0.001; as shown in **Table 2** and **Figure 4**).

Heterogeneity

The heterogeneity was calculated among all studies using the *Q-statistic* (*Q* > 0.05) and the I^2 statistic (I^2 = 0.0%). Heterogeneity was found in some groups, and the random-effects model was used.

Sensitivity analysis

A single study was deleted each time to investigate the influence of the individual dataset on the pooled *OR*s. The corresponding pooled *OR*s were not materially altered (data not shown), indicating that our results are statistically robust.

Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias of the literature. We found no asymmetry of the funnel plot, suggesting that there was no publica-

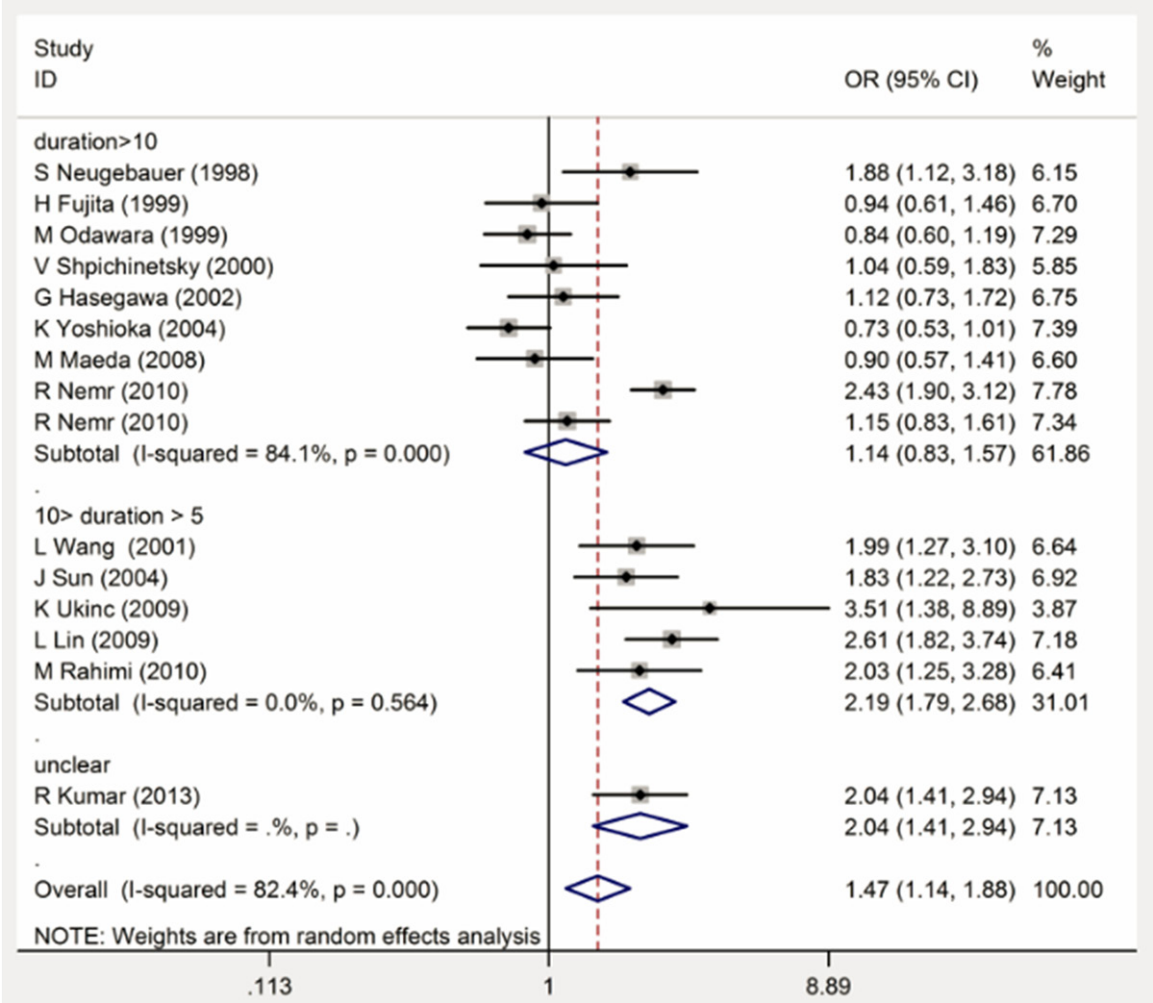

Figure 4. Forest plot of the MTHFR C677T polymorphism and DN stratified by average duration of diabetes (T allele vs C allele).

tion bias in the studies used in our meta-analysis.

Discussion

Diabetic nephropathy (DN) is a major contributor to the high mortality of patients with DM [18]. Several acquired risk factors, such as abnormal lipoprotein metabolism, hypertension, and hyperglycemia, have been identified in the development of DN [19]. Genetic susceptibility is thought to contribute to the pathogenesis of this complication. Studies of patients with type 2 DM have shown either that the *MTHFR* 677 T allele is a risk factor for DN or that no association between *MTHFR* C677T mutation and DN exists in Asian populations (shown in **Table 1**). Mutations of MTHFR gene together with elevated plasma homocysteine were shown to be associated with predisposition to developing type 2 DM complications, including DN. The results of our study suggest that the *MTHFR* 677 T allele is more likely to increase the risk of DN in Asian, West Asian, and Chinese populations, but we did not find this association in East Asian and Japanese populations. In our opinion, this inconsistency may be caused by two reasons: diabetes duration and ethnicity. It is noteworthy that the prevalence of 677T/T among Bahrainis (2.0%) was lower than that in Caucasians, and a north-south gradient in its prevalence has been described, supporting the ethnic contribution of 677T/T to DN risk [14]. We divided the 14 selected reports into 2 subgroups based on diabetes duration in the subjects: the "> 10 year duration" group and the "5-10 year duration" group. Nine reports are included in the ">

10 year duration" group and 5 reports are included in the "5-10 year duration" group. Interestingly, all 5 reports in "5-10 year duration" group support the conclusion that the *MTHFR* 677 T allele is more likely to increase the risk of DN. But only 2/9 reports in "> 10 year duration" group supported this conclusion (as shown in **Figure 4**). This finding suggests that *MTHFR* C677 T may play an important role in DN development in the early stages of type 2 DM. With increasing DM duration, other factors may contribute to risk of DN, thus diluting the influence of the *MTHFR* C677 T mutation.

There are some limitations to this study. Firstly, publication bias may have occurred, even though we did not detect this with our statistical tests. In order to avoid database bias, we only searched high-quality databases, such as Medline, PubMed, Embase, and Web of Science. Some databases in Chinese are excluded for 2 reasons: (1) most publications indexed in Chinese are of low quality; (2) this paper focuses on Asian populations, and including too many Chinese publications might bias the pooled results. Secondly, a meta-analysis essentially retains the methodological deficiencies of the included studies. Finally, this meta-analysis is based on unadjusted estimates, while a more precise analysis could be performed if individual data were available.

Conclusion

In conclusion, in spite of several limitations mentioned above, this meta-analysis suggests that the *MTHFR* C677T mutation increased the risk of DN, especially in early type 2 DM.

Disclosure of conflict of interest

None.

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Table S1. Scale for quality assessment

Criteria	Score
Representativeness of cases	
Selected from nephropathy registry or multiple nephropathy center sites	2
Selected from nephropathy department or nephropathy institute	1
Selected without clearly defined sampling frame or with extensive inclusion/exclusion criteria	0
Source of controls	
Population or community based	2
Both population-based and hospital-based/healthy volunteers blood donors	1.5
Hospital-based controls without DN	1
DN-free controls without total description	0.5
Not described	0
Ascertainment of DN	
Histologically or pathologically confirmed	2
Diagnosis of DN by patient medical record	1
Not described	0
Sample size	
> 1000	2
200-1000	1
< 200	0
Quality control of genotyping methods	
Clearly described a different genotyping assay to confirm the data	1
Not described	0
Hardy-Weinberg equilibrium	
Hardy-Weinberg equilibrium in controls	1
Hardy-Weinberg disequilibrium in controls	0.5
No checking for Hardy-Weinberg disequilibrium	0