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Association between the methylenetetrahydrofolate reductase (*MTHFR*) gene 677C>T and 1298A>C polymorphisms and the risk of diabetic nephropathy; a meta-analysis

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ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) is involved in the homocysteine metabolism. Two common variants of *MTHFR* gene (677C>T and 1298A>C), have been reported to reduce the MTHFR enzyme activity and leading to plasma hyperhomocysteinemia. There are a number of recent case-control studies that investigated the association between the *MTHFR* polymorphism and diabetic nephropathy (DN), albeit with inconsistent results. The aim of this meta-analysis is to evaluate the associations between the genetic polymorphisms of *MTHFR* with susceptibility to DN. A literature search was conducted on PubMed, Embase and Google scholar from inception till March 18, 2019. For *MTHFR* 677C>T analysis, a total of 23 studies including DM controls (3095 cases and 3187 DM controls) and 12 studies including non-DM controls (1590 cases and 2052 non-DM controls) were taken. For *MTHFR* 1298A>C analysis, a total of 7 studies using DM controls (959 cases and 1209 DM controls) and 3 studies using non-DM controls (400 cases and 802 non-DM controls) were taken. Meta-analysis showed that mutant genotypes of the 677C>T (OR: 1.58; 95%CI: 1.16-2.14) and 1298A>C (OR: 1.38; 95%CI: 1.16-1.65) polymorphisms in the *MTHFR* gene were associated with increased risk of DN (diabetic kidney disease). *MTHFR* 677C>T and 1298A>C polymorphisms revealed significant heterogeneity between studies. Further, there was no evidence for publication bias for these polymorphisms. In conclusion, this meta-analysis provides strong evidence that *MTHFR* 677C>T and 1298A>C polymorphisms may be associated with increased risks of DN. However, further studies are still needed to accurately determine whether *MTHFR* genetic polymorphisms are associated with susceptibility to DN.

Implication for health policy/practice/research/medical education:
Diabetic nephropathy (DN) (diabetic kidney disease) is the leading cause of chronic kidney disease and is characterized by the albuminuria. Homocysteine levels have been found elevated in patients with DN. The methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in the homocysteine metabolism. *MTHFR* gene polymorphisms were analyzed in many studies, but their results are inconclusive. We performed a meta-analysis of MTHFR 677C>T and 1298A>C studies. Our results support the association of MTHFR 677C>T and 1298A>C variants with the risk of DN.

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Introduction

Diabetes mellitus (DM) is a heterogeneous condition characterized by persistent hyperglycemia (1). Type 2 diabetes accounts for nearly 90%–95% of those with diabetes worldwide. Uncontrolled diabetes in synergy with the other metabolic aberrations is a serious health issue due to the morbidity associated with it (2). Several lines of evidences demonstrated that diabetes negatively affects the macro and microvasculature of various organs leading to the development of life-threatening health complications (3). The vascular complications include, microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (coronary artery disease, peripheral vascular disease, and stroke) complications that increase the risk for cardiovascular diseases (4). Diabetic nephropathy (DN) (diabetic kidney disease)is the leading

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cause of chronic kidney disease and is characterized by albuminuria (greater than 300 mg/dL), hypertension and a persistent decline in glomerular filtration rate leading to renal failure (5). Development and progression of nephropathy in diabetes patients seems to result from the interaction of genetic susceptibility with metabolic and hemodynamic changes (6).

The etiology of DN is multi-factorial and involves both environmental and genetic factors. Microalbuminuria is considered to reflect an early stage in the process of DN, and it may occur because of endothelial dysfunction due to hyperhomocysteinemia. Previous studies demonstrated that the elevated plasma levels of homocysteine (Hcy) have been related to insulin resistance and DN (7). MTHFR is an enzyme involved in the conversion of Hcy to methionine and catalyzes the reduction reaction of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate (8). MTHFR enzyme plays a vital role in folate metabolism and is involved in DNA synthesis, DNA repair and DNA methylation (9). The gene coding for *MTHFR* is located on chromosome 1 (1p36.3) and possessing 677C>T and 1298A>C polymorphisms that contribute to the inactivation of this enzyme (10). Several studies have examined the association between *MTHFR* gene polymorphisms and DN risk, however, the available proof reported to date is inconclusive (11-15). We performed the meta-analysis to investigate the relationship between *MTHFR* gene polymorphisms and DN susceptibility. In this meta-analysis, pooled estimate of the association under different genetic models for both 677C>T and 1298A>C polymorphisms were obtained. In addition, the heterogeneity between studies and the existence of publication bias were studied to understand the reliability of this purported association.

Materials and Methods

Literature search approach

A search for literature published in the English language was conducted until March 18, 2019, using the PubMed database, the Embase database, and Google Scholar with the following search terms: ('Diabetic nephropathy", "*MTHFR*", "677C>T", "1298A>C", "rs1801133", "rs1801131", "SNP", "polymorphism", and "genetic variant"). In addition, we reviewed the reference lists of retrieved papers and recent reviews. A total of 24 articles were found using the above search terms. Articles were excluded if they had the following characteristics; (*i*) review articles, (*ii*) non-epidemiologic articles, (*iii*) no DN subjects (*iv*) published in other than the English language and (*v*) did not include the SNPs 677C>T and 1298A>C. The present meta-analysis was conducted as per the guidelines issued in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis 2009 (16).

Data abstraction

All articles were evaluated independently by two reviewers (AG and SS) who extracted data that included the first author, publication year, geographic areas, ethnicity of study population, number of healthy controls, DM patients, DN patients, and genotypes of *MTHFR* 677C>T and 1298A>C polymorphisms. To test the population stratification in the controls, a chi-square test was applied to determine if *MTHFR* 677C>T genotype distribution in the controls conformed to Hardy–Weinberg equilibrium.

Statistical analysis

All analysis were performed using comprehensive meta-analysis is a software package version 2. Heterogeneity across individual studies was assessed by Cochran's Q test and I^2 statistic. The strength of the association between *MTHFR* polymorphisms and DN risk was measured by the pooled OR with its corresponding 95% CI. In the present meta-analysis, strength of the association was assessed in two different (allelic and dominant) genetic models. Further sensitivity analysis was conducted by excluding one study in each analysis to examine robustness of the method used for the meta-analysis. Potential publication bias was assessed by Begg's funnel plot and Egger's test.

Results

Study characteristics

The literature search strategy and process for the selection of papers for this meta-analysis is shown in Figure 1. A total of 24 articles with 25 studies that analyzed DM controls or non-DM controls or both were included in the final analysis (9,11-15,17-35). Out of these only 8 articles studied both *MTHFR* 677C>T and 1298A>C polymorphisms. Further, one of the included article that had included two populations was considered as two independent studies (28). For *MTHFR* 677C>T analysis, a total of 23 studies including DM controls (3095 cases and 3187 DM controls) and 12 studies including non-DM controls (1590 cases and 2052 non-DM controls)

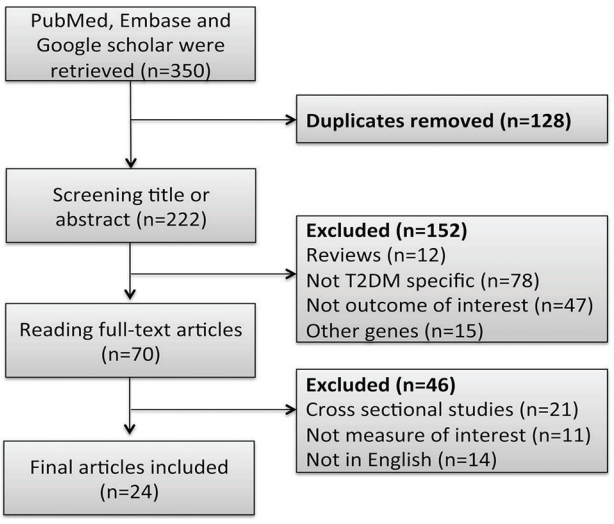

Figure 1. Identification process for eligible studies.

were taken. For *MTHFR* 1298A>C analysis, a total of 7 studies including DM controls (959 cases and 1209 DM controls) and 3 studies including non-DM controls (400 cases and 802 non-DM controls) were taken. The number of patients and controls, genotypes, HWE and other study characteristics of each study included in the meta-analysis are listed in Tables 1 and 2.

Heterogeneity

When we chose patients with DM as controls or non-DM subjects as controls, the association between 677C>T polymorphism and DN showed a significant heterogeneity between studies in both allelic ($P<0.001$, I^2 >0.86) and dominant model ($P<0.001$, $I^2>0.86$). In the subgroup analysis according to ethnicity, there still existed heterogeneity in both genetic models for 677C>T polymorphism. Nevertheless, the association between 1298A>C polymorphism and DN, significant heterogeneity between studies was found only in allelic model ($P=0.026$, I^2 0.58) when we chose patients with DM as controls (Tables 1 and 2).

Association of the MTHFR polymorphisms with DN and subgroup analysis when compared with DM patients

The meta-analysis results showed that there was a statistically significant association between *MTHFR* 677C>T and DN risk in allelic (OR: 1.45; 95% CI: 1.16-1.81) and dominant genetic models (OR: 1.58; 95%CI: 1.16-2.14) (Figure 2A, Table 3). In the subgroup analysis according to ethnicity, there still existed significant association between *MTHFR* 677C>T and DN risk for Caucasian population (OR: 1.68; 95% CI: 1.12-2.54). The *MTHFR* 1298A>C is associated with DN risk only in dominant genetic model (OR: 1.38; 95% CI: 1.16-1.65). Subgroup analysis showed that this association exists only in Caucasian populations (OR: 1.32; 95% CI: 1.10-1.59) (Table 3, Figure 3A).

Association of the MTHFR polymorphisms with DN and subgroup analyses when compared with non-DM controls

As listed in Table 3, there was obviously significant association between *MTHFR* 677C>T and DN risk in allelic (OR: 1.98; 95% CI: 1.41-2.78) and dominant genetic models (OR: 2.35; 95% CI: 1.47-3.75) (Table 3, Figure 2B). In the subgroup analysis significant association between *MTHFR* 677C>T and DN risk was found only in allelic (OR: 2.16; 95 %CI: 1.67-2.79) and dominant genetic models (OR: 2.63; 95% CI: 1.90-3.62) of Asian populations. The *MTHFR* 1298A>C is associated with DN risk only in allelic model (OR: 1.35; 95% CI: 1.10-1.64). Subgroup analysis revealed association only in allelic (OR: 1.64; 95% CI: 1.14-2.35) and dominant genetic models

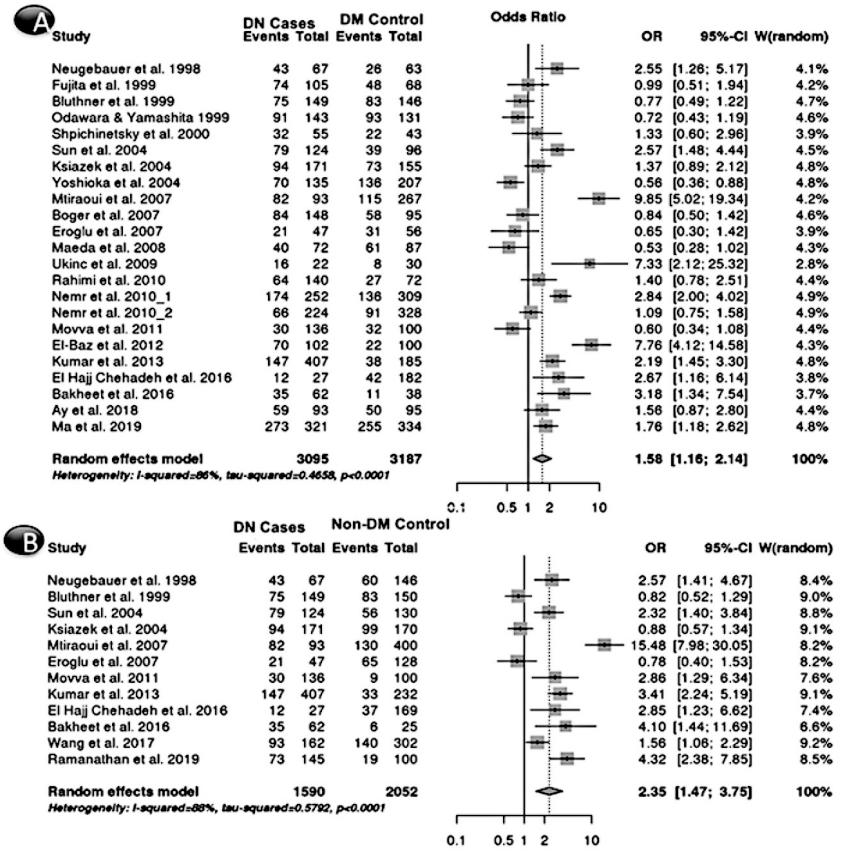

Figure 2. Meta-analysis for the association between DN risk and *MTHFR* 677C>T polymorphism.

Table 1. Distribution of *MTHFR* 677C>T genotypes and HWE *P* values of in the eligible studies

Reference	Ethnicity	Country	Genotyping	DN			DM control			Non-DM controls			HWE *P* values	
				CC	CT	TT	CC	CT	TT	CC	CT	TT	DC	NDC
Neugebauer et al. 1998 (17)	Asian	Japan	PCR-RFLP	24	31	12	37	18	8	86	43	17	0.167	0.011
Fujita et al. 1999 (18)	Asian	Japan	PCR-RFLP	31	57	17	20	39	9	-	-	-	0.468	-
Bluthner et al. 1999 (19)	Caucasian	Poland	PCR-RFLP	74	50	25	63	65	18	67	68	15	0.946	0.709
Odawara & Yamashita 1999 (20)	Asian	Japan	PCR-RFLP	52	65	26	38	68	25	-	-	-	0.750	-
Shpichinetsky et al. 2000 (21)	Caucasian	Israel	PCR-RFLP	23	22	10	21	16	6	-	-	-	0.569	-
Sun et al. 2004 (22)	Asian	China	PCR-RFLP	45	53	26	57	23	16	74	34	22	0.000	<0.001
Ksiazek et al. 2004 (23)	Caucasian	Poland	PCR-RFLP	77	65	29	82	58	15	71	83	16	0.569	0.356
Yoshioka et al. 2004 (24)	Asian	Japan	PCR-RFLP	65	52	18	71	107	29	-	-	-	0.569	-
Mtiraoui et al. 2007 (13)	Caucasian	Tunisia	PCR-RFLP	11	56	26	152	79	36	270	94	36	0.000	<0.001
Boger et al. 2007 (25)	Caucasian	Germany	PCR-RFLP	64	69	15	37	45	13	-	-	-	0.946	-
Eroglu et al. 2007 (26)	Caucasian	TURKEY	PCR-RFLP	26	20	1	25	25	6	63	58	7	0.946	0.295
Maeda et al. 2008 (15)	Asian	Japan	PCR-RFLP	32	25	15	26	51	10	-	-	-	0.214	-
Ukinc et al. 2009 (12)	Caucasian	TURKEY	Melt curve	6	16	0	22	8	0	-	-	-	0.574	-
Rahimi et al. 2010 (27)	Caucasian	Iran	PCR-RFLP	76	62	2	45	26	1	-	-	-	0.499	-
Nemr et al. 2010_1 (28)	Caucasian	Lebanon	PCR-RFLP	78	104	70	173	100	36	-	-	-	0.005	-
Nemr et al. 2010_2 (28)	Caucasian	Bahrain	PCR-RFLP	158	58	8	237	86	5	-	-	-	0.574	-
Movva et al. 2011 (11)	Asian	India	PCR-RFLP	106	30	0	68	32	0	91	9	0	0.218	0.696
El-Baz et al. 2012 (14)	African	Egypt	PCR-RFLP	32	46	24	78	19	3	-	-	-	0.499	-
Kumar et al. 2013 (29)	Asian	India	PCR-RFLP	260	129	18	147	35	3	199	29	4	0.750	0.057
El Hajj Chehadeh et al. 2016 (30)	Caucasian	UAE	PCR-RFLP	15	10	2	140	39	3	132	27	10	0.946	0.000
Bakheet et al. 2016 (31)	African	Egypt	PCR-RFLP	27	27	8	27	9	2	19	5	1	0.569	0.476
Wang et al. 2017 (32)	Asian	China	PCR-RFLP	69	72	21	-	-	-	162	127	13	0.574	0.105
Ay et al. 2018 (33)	Caucasian	Turkey	PCR-RFLP	34	43	16	45	38	12	-	-	-	-	-
Ramanathan et al. 2019 (34)	Asian	India	PCR-RFLP	72	71	2	-	-	-	81	19	0	0.946	0.392
Ma et al. 2019 (35)	Asian	China	PCR-RFLP	48	166	107	79	169	86	-	-	-	-	-

Table 2. Distribution of *MTHFR* 1298A>C genotypes and HWE *P* values of in the eligible studies

Author, year	Ethnicity	Country	Genotyping	DN			DM Control			non-DM Controls			HWE P values	
				AA	AC	CC	AA	AC	CC	AA	AC	CC	DC	NDC
Shpichinetsky et al. 2000 (21)	Caucasian	Israel	PCR-RFLP	26	19	10	24	9	10	-	-	-	0.002	-
Mtiraoui et al. 2007 (13)	Caucasian	Tunisia	PCR-RFLP	41	50	2	150	90	27	256	128	16	0.043	1.0
Nemr et al. 2010_1 (28)	Caucasian	Lebanon	PCR-RFLP	100	120	32	135	133	41	-	-	-	0.513	-
Nemr et al. 2010_2 (28)	Caucasian	Bahrain	PCR-RFLP	96	113	15	142	147	39	-	-	-	0.919	-
Rahimi et al. 2010 (27)	Caucasian	Iran	PCR-RFLP	52	56	32	41	22	8	-	-	-	0.136	-
El-Baz et al. 2012 (14)	African	Egypt	PCR-RFLP	53	41	8	69	27	4	-	-	-	0.602	-
Wang et al. 2017 (32)	Asian	China	PCR-RFLP	81	69	12	-	-	-	163	123	16	-	0.361
Ay et al. 2018 (33)	Caucasian	Turkey	PCR-RFLP	20	71	2	29	60	2	-	-	-	<0.001	-
Ramanathan et al. 2019 (34)	Asian	India	PCR-RFLP	57	66	22	-	-	-	44	50	6	-	0.275

(OR: 2.26; 95% CI: 1.43-3.56) of Caucasian populations (Table 3, Figure 3B).

Sensitivity analysis and publication bias

The analysis results suggested that no individual studies significantly affected the pooled DN risk of both *MTHFR* 677C>T and 1298A>C variants (Figures 4A-B and 5A-B), indicating a statistically robust result. The shapes of the funnel plots seemed symmetrical for both *MTHFR* 677C>T (Figure 6A) and *MTHFR* 1298A>C (Figure 6B) polymorphisms indicating the absence of publication bias. Egger's test also showed that there was no strong statistical evidence of publication bias for both *MTHFR* 677C>T and 1298A>C polymorphisms in all genetic models ($P > 0.050$).

Discussion

About 25%-40% of patients with diabetes are susceptible to develop kidney disease. Although inadequate glycemic control is one of the fundamental risk factors for the development nephropathy, it is not an inevitable complication of diabetes. The involvement of multiple risk factors such as duration and severity of diabetes, environmental factors, life-style stressors and genetic factors make it difficult to determine a person's risk of inheriting nephropathy. Familial aggregation of DN is one of the major evidence for a heritable genetic susceptibility to DN. However, despite long and intensive research efforts to determine the causative genetic components remain elusive (36-38).

Our meta-analysis revealed a significant association

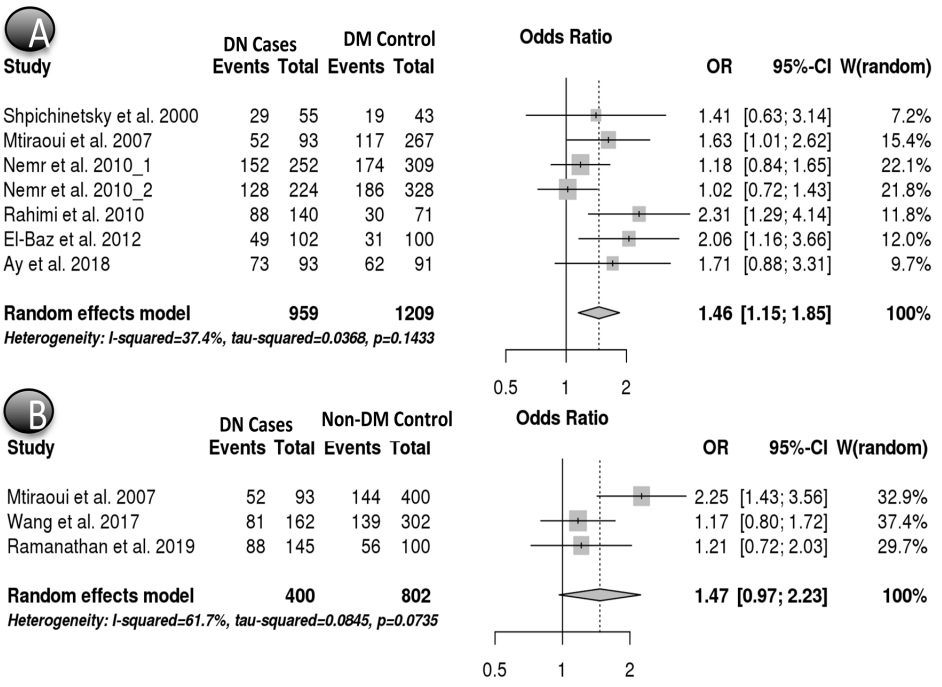

Figure 3. Meta-analysis for the association between DN risk and *MTHFR* 1298A>C polymorphism. A: comparison with DM controls; B: comparison with non-DM controls.

Table 3. The heterogeneity results for the association of *MTHFR* 677C>T gene polymorphisms and DN in different genetic models

***MTHFR* 677C>T**	**DN vs. DM patients**				**DN vs. non-DM control**			
	Overall	**By ethnicity**			**Overall**	**By ethnicity**		
		African	**Asian**	**Caucasian**		**African**	**Asian**	**Caucasian**
Number of studies	23	2	9	12	12	1	6	5
Allele contrast (T vs. C)								
I^2	0.86	0.73	0.78	0.84	0.87	NA	0.53	0.94
$P_{Heterogeneity}$	<0.001	0.055	<0.0011	<0.001	<0.001	NA	0.058	<0.001
OR	1.45a	4.07a	1.16a	1.48a	1.98a	3.26b	2.16a	1.60a
95% CI	(1.16- 1.81)	(1.79-9.28)	(0.89- 1.52)	(1.09-2.00)	(1.41-2.78)	(1.35-7.86)	(1.67- 2.79)	(0.76-3.35)
Dominant model (TT+CT vs. CC)								
I^2	0.86	0.63	0.84	0.85	0.88	NA	0.56	0.94
$P_{Heterogeneity}$	<0.001	0.102	<0.001	<0.001	<0.001	NA	0.046	<0.001
OR	1.58a	5.68b	1.14a	1.68a	2.35a	4.10b	2.63a	1.87a
95% CI	(1.16- 2.14)	(3.41- 9.46)	(0.74- 1.77)	(1.12- 2.54)	(1.47- 3.75)	(1.44- 11.69)	(1.90- 3.62)	(0.65- 5.32)
***MTHFR* 1298A>C**	Overall	African	Asian	Caucasian	Overall	African	Asian	Caucasian
Number of studies	7	1	0	6	3	0	2	1
Allele contrast (C vs. A)								
I^2	0.58	NA	-	0.52	0	-	0	NA
$P_{Heterogeneity}$	0.026	NA	-	0.063	0.369	-	0.536	NA
OR	1.23a	1.83b	-	1.16b	1.35b	-	1.24b	1.64b
95% CI	(0.99- 1.53)	(1.14- 2.94)	-	(0.94- 1.44)	(1.10- 1.64)	-	(0.98- 1.57)	(1.14- 2.35)
Dominant model (CC+AC vs. AA)								
I^2	0.37	NA	-	0.34	0.62	-	0	NA
$P_{Heterogeneity}$	0.143	NA	-	0.184	0.074	-	0.918	NA
OR	1.38b	2.06b	-	1.32b	1.47a	-	1.19b	2.26b
95% CI	(1.16- 1.65)	(1.16- 3.66)	-	(1.10- 1.59)	(0.97- 2.23)	-	(0.87- 1.61)	(1.43- 3.56)

[a] Random effect model; [b] fixed effect model.

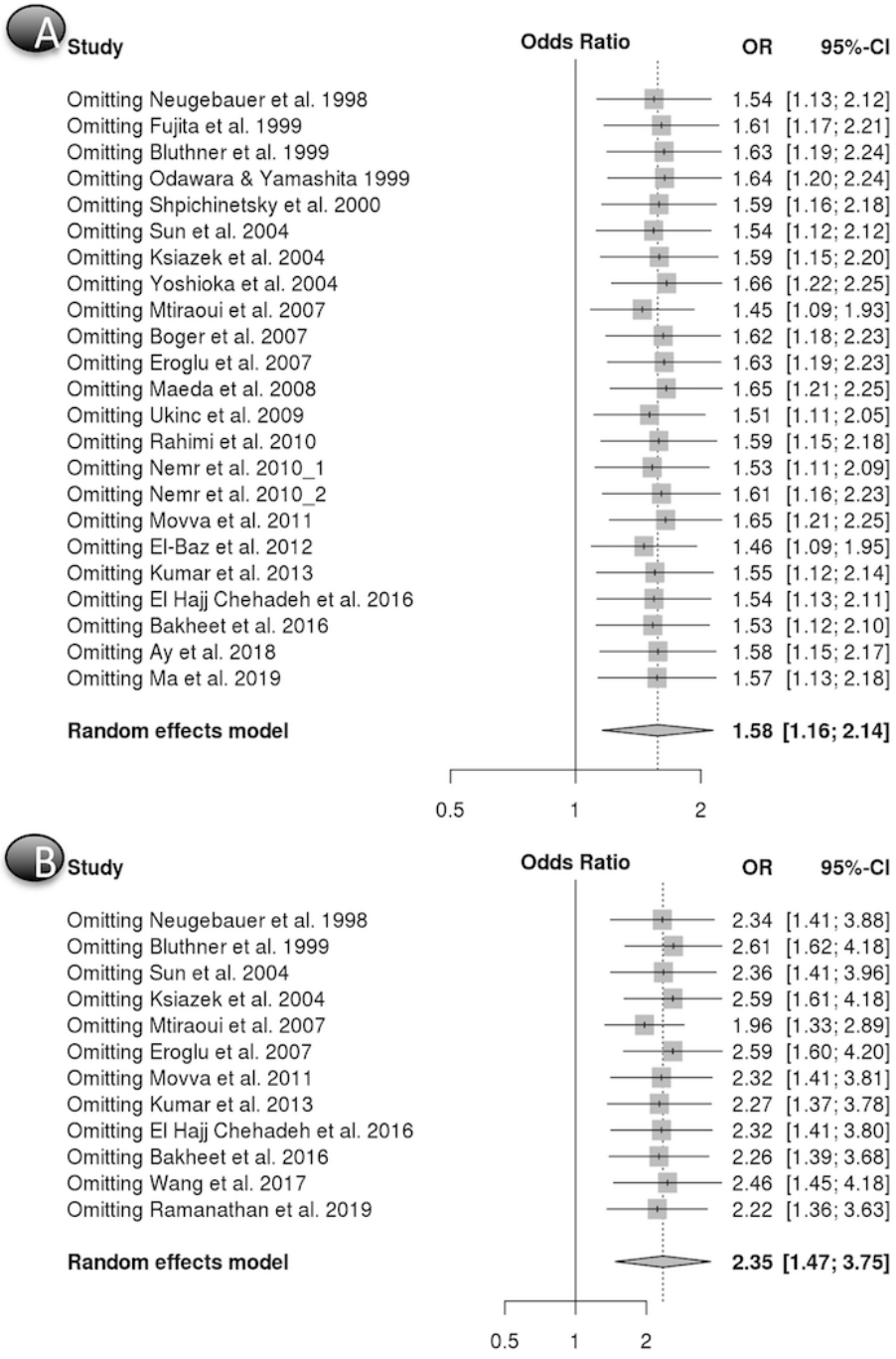

Figure 4. Sensitivity analysis of meta-analysis results of MTHFR 677C>T polymorphism. A: comparison with DM controls; B: comparison with non-DM controls.

between *MTHFR* polymorphisms and DN risk. Meta-analysis of *MTHFR* polymorphisms revealed significant heterogeneity between studies for both 677C>T and 1298A>C. Further, there was no evidence for publication bias for these polymorphisms. In consistent with our results, a series of earlier published meta-analyses provided evidence of association between *MTHFR* 677C>T polymorphism and DN risk in various ethnicities (39-45). With regards to *MTHFR* 1298A>C only one meta-analysis performed in which no association between *MTHFR* 1298A>C and DN risk was observed (44). Higher levels of homocysteine in patients with DN were demonstrated in multiple studies (46-48). This is further supported by presence of increased erythrocyte S-Adenosyl-L-homocysteine, decreased erythrocyte S-Adenosyl methionine and lymphocyte MTHFR activity in patients with advanced nephropathy (49). Compared to subjects without complications, individuals with DN and

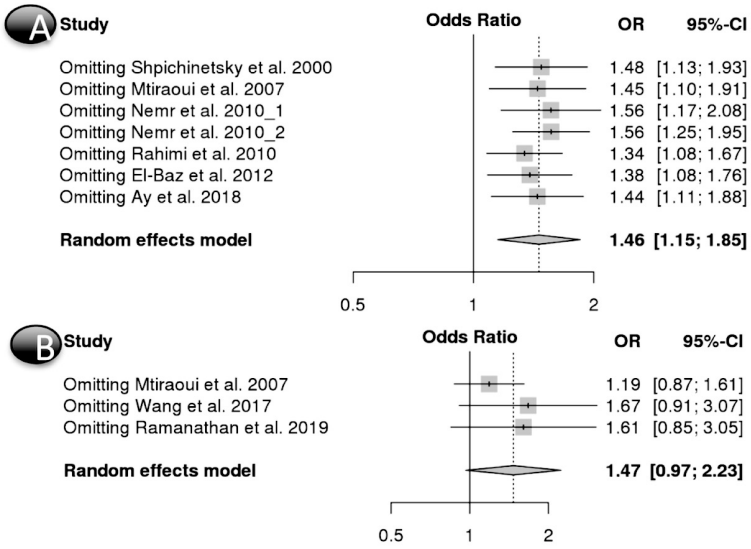

Figure 5. Sensitivity analysis of meta-analysis results of *MTHFR* 1298A>C polymorphism. A: comparison with DM controls; B: comparison with non-DM controls.

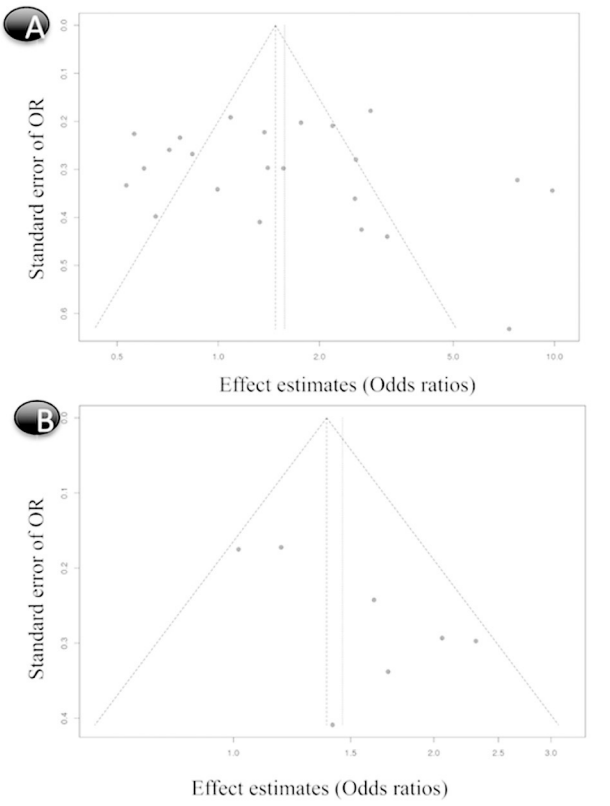

Figure 6. Funnel plot used in assessing publication bias in the meta-analysis. A: *MTHFR* 677C>T polymorphism; B: MTHFR 1298A>C polymorphism.

hypermethylated profile in the *MTHFR* gene promoter showed higher levels of alpha-1 acid glycoprotein and total antioxidant capacity (50). In individuals carrying 677CC/1298AA haplotype, the hypermethylated profile was linked with higher fasting glycemia values (51). Further, a direct link between perturbations in 1-carbon metabolism, through an interaction of total homocysteine and the activity of *MTHFR* enzyme on epigenetic regulation of the genome via DNA methylation (52).

Conclusion

In summary, this meta-analysis provided strong evidence for association between *MTHFR* 677C>T and 1298A>C polymorphisms and DN risk. As the majority of studies included in this meta-analysis were Caucasians and East Asians and that the distribution of *MTHFR* genetic polymorphism differs among ethnic groups, the results cannot be extrapolated to patients belonging to any other ethnic groups. However, further studies are still needed to accurately determine whether *MTHFR* genetic polymorphisms are associated with susceptibility to DN.

Authors' contribution

Study Conceived; BVKSL. Data collected; AG, SS and SL. Data analyzed; BVKSL. Wrote the paper; AG, SS, SL and BVKSL. All authors have seen and approved the manuscript.

Conflicts of interest

There are no conflicts of interests.

Ethical considerations

The authors of this manuscript declare that they all have followed the ethical requirements for this communication. Also, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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