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RESEARCH ARTICLE

Genetics of diabetic neuropathy: Systematic review, meta-analysis and trial sequential analysis

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Abstract

Objective: Diabetic neuropathy (DN) is one of the most common complications of diabetes that occurs in more than 67% of individuals with diabetes. Genetic polymorphisms may play an important role in DN development. However, until now, the association between genetic polymorphisms and DN risk has remained unknown. We performed a systematic review, meta-analysis, and trial sequential analysis (TSA) of the association between all genetic polymorphisms and DN risk. **Methods**: Relevant published studies examining the relationship between all genetic polymorphisms and DN were obtained based on a designed search strategy up to 28 February 2019. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess overall pooled effects of genetic models as well as in subgroup analyses. Sensitive analysis and publication bias were applied to evaluate the reliability of the study. Moreover, TSA was conducted to estimate the robustness of the results. **Results**: We conducted a systematic review of a total of 1256 articles, and then 106 publications reporting on 136 polymorphisms of 76 genes were extracted. We performed 107 meta-analyses on 36 studies involving 12,221 subjects to derive pooled effect estimates for eight polymorphisms. We identified that ACE I>D, MTHFR 1298A/C, GPx-1 rs1050450, and CAT -262C/T were associated with DN, while MTHFR C677T, GSTM1, GSTT1, and IL-10 -1082G/A were not. Sensitivity analysis, funnel plot, and Egger's test displayed robust results. Furthermore, the results of TSA indicated sufficient sample size in studies of ACE, GPx-1, GSTM1, and IL-10 polymorphisms. **Interpretation**: Our study assessed the association between ACE I>D, MTHFR C677T, MTHFR 1298A/C, GPx-1 rs1050450, CAT -262C/T, GSTM1, GSTT1, and IL-10 -1082G/A polymorphisms and DN risk. We hope that the data in our research study are used to study DN genetics.

Introduction

As a global public threat, diabetes mellitus (DM) is a life-long disease that involves multiple organs and systems, and the morbidity of diabetes among adults could rise to 552 million by 2030.[1,2] As the most common complication of diabetes, diabetic neuropathy (DN) including diabetic autonomic neuropathy and somatic sensorimotor neuropathy has a prevalence of 8% in newly diagnosed diabetic patients and over 50% in patients with a long course of disease.[3,4] DN may produce a series of clinical manifestations including numbness, tingling, pain, and/or weakness which considerably decrease the quality of life in patients.[5] Currently, the risk factors and pathogenesis of DN have drawn increasing attention.

Many factors are known to be associated with DN susceptibility, including smoking, obesity, poor glycemic control, and duration of diabetes, but there are still some potential factors leading to the occurrence of DN, such as genetic variants.[6,7] In 1997, Vague P et al. first found an association between the ATP1 A1 gene polymorphism and DN risk.[8] Since then, an increasing number of studies have been carried out to investigate the association between various genetic polymorphisms and DN susceptibility, such as ACE I/D, MTHFR C677T and GSTM1.[9,10] For example, in 2012, Jurado et al.[11] reported that the ID genotype of the ACE I/D polymorphism had a protective effect on the development of DN. However, others drew a completely different conclusion in that the ID genotype may lead to an increased DN risk.[2,12] Similarly, a significant association between the MTHFR gene C677T mutation and DN was observed by Yigit in 2013,[13] which could not be replicated by Russo in 2016.[14]

Till now, the findings of individual studies were not always consistent, and no systematic review covered all genetic polymorphisms has been reported. To fill this gap in medical literature worldwide, we performed the first systematic review and meta-analysis involving all the available evidence in the field of genetic variants and DN susceptibility.

Materials and Methods

Search strategy

A comprehensive literature search was performed in the PubMed and Embase databases up to 28 February 2019, using the following terms: "diabetic neuropathy/diabetic polyneuropathy/diabetic peripheral neuropathy/DPN/cardiovascular autonomic neuropathy/CAN" and "polymorphism/variant/genotype/allele/SNP/mutation". As a complement, we also checked the reference list of the meta-analyses and review articles on genetic association for DN, in case the references they used had been missed in original search.

Inclusion criteria

Studies were included if they met the following conditions: (1) case–control studies; (2) for the association between any genetic polymorphism and DN susceptibility; (3) sufficient allele and genotype data to calculate the odds ratios (ORs) with 95% confidence intervals (CIs); (4) studies published in English. If two papers included the same dataset, but one included additional data not found in the other paper, only the later was included. Any genetic polymorphism with three or more published studies was included in our meta-analysis.

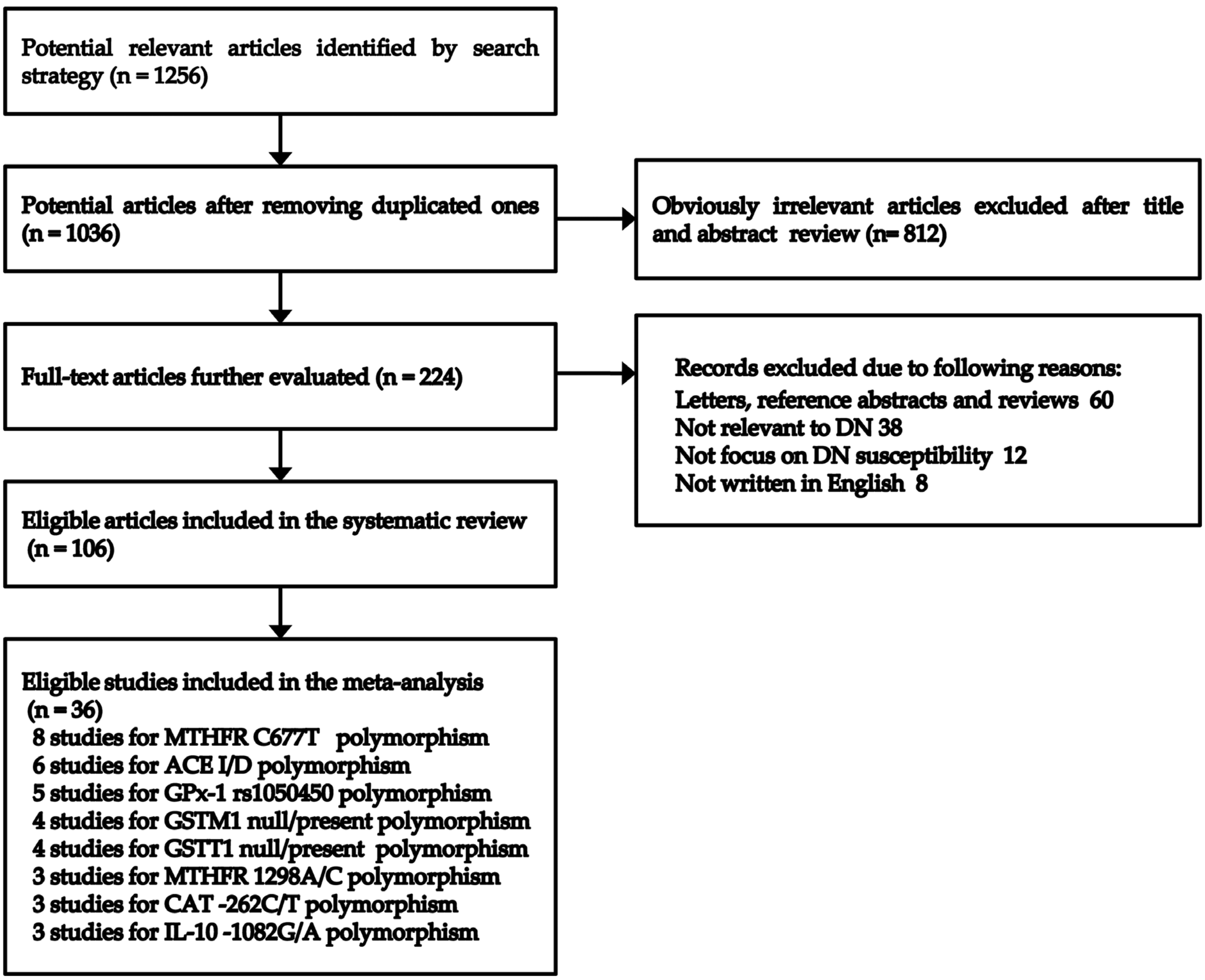

Figure 1. Flow diagram of the study selection process.

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

First author	Year	Region	Ethnicity	Case	Control	Genotype distribution						Genotyping method	Age and gender matched	Type of diabetes	Type of DN	NOS	*P* for HWE[1]
						Case			Control								
ACE I>D						II	ID	DD	II	ID	DD						
Inanir, A.	2013	Turkey	Asian	235	281	43	91	101	63	123	95	NA	Matched	T1DM & T2DM	DN	7	0.058
Mansoor, Q.	2012	Pakistan	Asian	276	496	59	161	56	161	230	105	NA	NA	T2DM	DN	5	0.177
Stephens, J. W.	2006	UK	Caucasian	173	399	25	87	61	78	199	125	NA	Matched	T2DM	DN (sensorimotor)	6	0.940
Costacou, T.	2006	USA	Caucasian	114	256	86		28	200		56	NA	Matched	T1DM	DN (sensorimotor)	7	NA
Degirmenci, I.	2005	Turkey	Asian	65	75	6	38	21	19	35	21	NA	NA	T2DM	DN	5	0.568
Ito, H.	2002	Japan	Asian	21	63	14	6	1	26	27	10	NA	Matched	T2DM	DN (sensorimotor)	7	0.506
MTHFR C677T						CC	CT	TT	CC	CT	TT						
Kakavand Hamidi, A.	2018	Iran	Asian	141	107	73	62	6	53	42	12	PCR-RFLP	Matched	T2DM	DN (sensorimotor)	7	0.408
Jimenez-Ramirez, F. J.	2017	Puerto Rico	Caucasian	89	400	72	8	9	184	159	57	PCR-RFLP	Matched	T2DM	DN	7	0.020
Fekih-Mrissa, N.	2017	Tunisia	Caucasian	16	144	4	12	0	52	90	2	NA	Matched	T2DM	DN	7	0.000
Russo, G. T.	2016	Italy	Caucasian	79	184	27	52		51	133		NA	Unmatched	T2DM	DN (sensorimotor)	5	NA
Yigit, S.	2013	Turky	Asian	230	282	123	85	22	180	93	9	PCR-RFLP	Matched	T1DM & T2DM	DN (sensorimotor)	8	0.469
Wang, H.	2012	China	Asian	101	149	20	50	31	28	100	21	PCR-RFLP	Matched	T2DM	DN	7	0.000
Costacou, T.	2006	USA	Caucasian	114	256	47	67		88	168		NA	Matched	T1DM	DN (sensorimotor)	7	NA
Ambrosch, A.	2001	German	Caucasian	43	22	15	25	2	8	12	2	PCR-RFLP	Matched	T2DM	DN	7	0.402
MTHFR 1298A/C						AA	AC	CC	AA	AC	CC						
Kakavand Hamidi, A.	2018	Iran	Asian	118	106	68	47	3	67	39	0	PCR-RFLP	Matched	T2DM	DN (sensorimotor)	7	0.020
Jimenez-Ramirez, F. J.	2017	Puerto Rico	Caucasian	89	400	41	43	1	251	138	11	PCR-RFLP	Matched	T2DM	DN	7	0.118
Fekih-Mrissa, N.	2017	Tunisia	Caucasian	16	144	10	6	0	82	42	20	NA	Matched	T2DM	DN	7	0.001
GPx-1 rs1050450						CC	CT	TT	CC	CT	TT						
Buraczynska, M.	2017	Poland	Caucasian	406	838	167	179	60	468	281	89	NA	Matched	T2DM	DN	7	0.000
Tang, T. S.-a	2012	UK	Caucasian	211	558	79	108	24	265	224	69	PCR-RFLP	Matched	T1DM & T2DM	DN (sensorimotor)	6	0.047
Tang, T. S.-b	2012	UK	Caucasian	63	319	22	38	3	163	137	19	PCR-RFLP	Matched	T1DM & T2DM	DN (sensorimotor)	6	0.160
Matsuno, S.-a	2011	Japan	Asian	79	94	62	17	0	87	7	0	PCR-RFLP	Matched	T2DM	DN (sensorimotor)	7	0.708
Matsuno, S.-b	2011	Japan	Asian	25	148	22	3	0	127	21	0	PCR-RFLP	Matched	T2DM	DN (DAN)	7	0.353
CAT −262C/T						TT	CT	CC	TT	CT	CC						
Snahnicanova, Z.	2018	Slovak	Caucasian	34	80	1	13	20	6	32	42	TaqMan	Matched	T1DM	DN (sensorimotor)	6	0.978
Kasznicki, J.	2016	Poland	Caucasian	100	129	4	30	66	7	43	79	PCR-RFLP	Matched	T2DM	DN (sensorimotor)	6	0.719
Babizhayev, M. A.	2015	Russia	Caucasian	216	250	53	80	83	96	74	80	NA	Matched	T1DM	DN	7	0.000
GSTM1 null/present						Null	Present		Null	Present							
Stoian, A.	2015	Romania	Caucasian	42	42	18	24		22	20		PCR-RFLP	Matched	T2DM	DN (sensorimotor)	6	NA
Babizhayev, M. A	2015	Russia	Caucasian	216	250	278	154		344	156		NA	Matched	T1DM	DN	7	NA
Zaki, M. A.	2015	Egypt	Caucasian	27	27	10	13		3	1		NA	Matched	T2DM	DN	6	NA

(*Continued*)

Table 1. Continued.

First author	Year	Region	Ethnicity	Case	Control	Genotype distribution						Genotyping method	Age and gender matched	Type of diabetes	Type of DN	NOS	*P* for HWE[1]
						Case			Control								
Vojtkova, J.	2013	Slovak	Caucasian	19	27	10		9	10		17	NA	Matched	T1DM	DN (DAN)	7	NA
GSTT1 null/present						Null		Present	Null		Present						
Stoian, A.	2015	Romania	Caucasian	42	42	7		35	8		34	PCR-RFLP	Matched	T2DM	DN (sensorimotor)	6	NA
Babizhayev, M. A.	2015	Russia	Caucasian	216	250	160		272	170		330	NA	Matched	T1DM	DN	7	NA
Zaki, M. A.	2015	Egypt	Caucasian	27	27	7		0	4		16	NA	Matched	T2DM	DN	6	NA
Vojtkova, J.	2013	Slovak	Caucasian	19	27	3		16	13		14	NA	Matched	T1DM	DN (DAN)	7	NA
IL-10-1082G/A						GG	GA	AA	GG	GA	AA						
Canecki-Varžić, S.	2018	Croatia	Caucasian	204	96	45	90	27	28	38	11	Taqman	NA	T2DM	DN	5	0.742
Rodrigues, K. F.	2015	Brazil	Caucasian	42	60	3	20	19	9	27	24	PCR-SSP	Unmatched	T2DM	DN (sensorimotor)	6	0.757
Kolla, V. K.	2009	India	Asian	198	202	32	42	124	13	41	148	ARMS PCR	Matched	T2DM	DN (sensorimotor)	6	0.000

DN, diabetic neuropathy; NOS, Newcastle-Ottawa Quality Assessment Scale; HWE, Hardy–Weinberg Equilibrium; NA, not available; PCR-RFLP, polymerase chain reaction and restriction fragment length polymorphism; PCR-SSP, polymerase chain reaction-sequence specific primers; ARMS PCR, amplification refractory mutation system polymerase chain reaction methods; DAN, diabetic autonomic neuropathy; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

[1]HWE in control.

Data extraction

By using a standardized form, two investigators independently extracted the following data: the name of the first author, publication year, region, ethnicity, sample size, allele and genotype frequencies, genotyping methods, age- and gender-matched status, type of diabetes, type of neuropathy, Newcastle-Ottawa Quality Assessment Scale (NOS) score, and *P* value for Hardy–Weinberg equilibrium (HWE) in the control group. The quality of studies was evaluated using the NOS and scores >5 were considered to be of high quality, otherwise, they were thought to be with low quality.

Meta-analysis

We used Stata 12.0 software to conduct the meta-analysis for each genetic polymorphism to determine the pooled ORs and 95% CIs. We calculated the pooled results under all five genetic models (allelic, recessive, dominant, homozygous, and heterozygous model). Heterogeneity was measured by the I^2 statistic, and $I^2 > 50\%$ was considered significant heterogeneity. The random-effects model was used if significant heterogeneity existed or else the fixed-effects model was adopted. Subgroup analyses were performed based on ethnicity, genotyping methods, age- and gender-matched status, HWE status of controls, quality of studies, source of control, type of diabetes, and type of neuropathy. The sensitivity analyses were conducted by sequentially omitting each study to detect the stability of pooled results and source of heterogeneity. Publication bias was explored using visual inspection of the funnel plot and Egger's test. $P < 0.05$ was considered to be statistically significant.

Trial sequential analysis

Meta-analysis may lead to a false-positive or negative conclusion.[15] Hence, we used trial sequential analysis (TSA) to reduce these statistical errors.[16] TSA is a novel statistical analysis method that uses a combination of techniques that provides required information size (RIS), a threshold of statistically significant effect, for evaluating whether sufficient evidence is included and whether a result is reliable or not, in meta-analysis. Additionally, a threshold of futility could be tested by TSA to find a conclusion of no effect before reaching the information size by using TSA software (version 0.9.5.10 beta) (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen, Denmark). We computed the RIS based on an alpha risk of 5%, a beta risk of 20%, a relative risk reduction of 20% and a two-sided boundary type. For those analyses that the *Z*-curve reached the RIS line or monitoring the boundary line or futility area, it indicates that enough samples are included in the studies, and their results are credible. Otherwise, the amount of information is not large enough, and more evidence is needed.

Results

Study selection

In total, 1256 articles were retrieved according to our search strategy. First, we excluded 1032 articles by duplicate screening as well as title and abstract reviewing. Second, after full-text reviewing, 118 studies containing 60 letters, reference abstracts and reviews; 38 studies not relevant to DN; 12 studies not focused on DN susceptibility; and eight studies not written in English were excluded. Third, 106 eligible articles were selected in our systematic review, and the relationship between all 136 genetic polymorphisms and DN susceptibility was extracted and listed in Table S1. Finally, for any polymorphism with three or more published studies and sufficient genotype data to extract, we keep it into our meta-analysis. A total of 36 studies were involved in the meta-analysis, and the entire process of study selection is shown in Figure 1.

Study characteristics

Thirty-six studies with 4515 cases and 7706 controls were included in the meta-analysis according to the inclusion and exclusion criteria.[13,14,17–39] The general characteristics of the studies are summarized in Table 1. Among the 36 studies, 6 were related to ACE I/D, 8 to MTHFR C677T, 3 to MTHFR 1298A/C, 5 to GPx-1 rs1050450, 3 to CAT -262C/T, 4 to GSTM1 and GSTT1 and 3 to IL-10 -1082G/A. In these studies, 26 studies were performed in the Caucasian population and 10 remaining studies were performed in the Asian population. The genotyping methods included polymerase chain reaction-restriction fragment length polymorphism, TaqMan, polymerase chain reaction-sequence specific primers, and amplification refractory mutation system-polymerase chain reaction. For the quality of studies, all of them except four[14,18,21,37] scored more than 5 in NOS. In addition, for the HWE of controls, most of the articles met HWE equilibrium, while 10 studies failed.[24–26,30–,32,37,39]

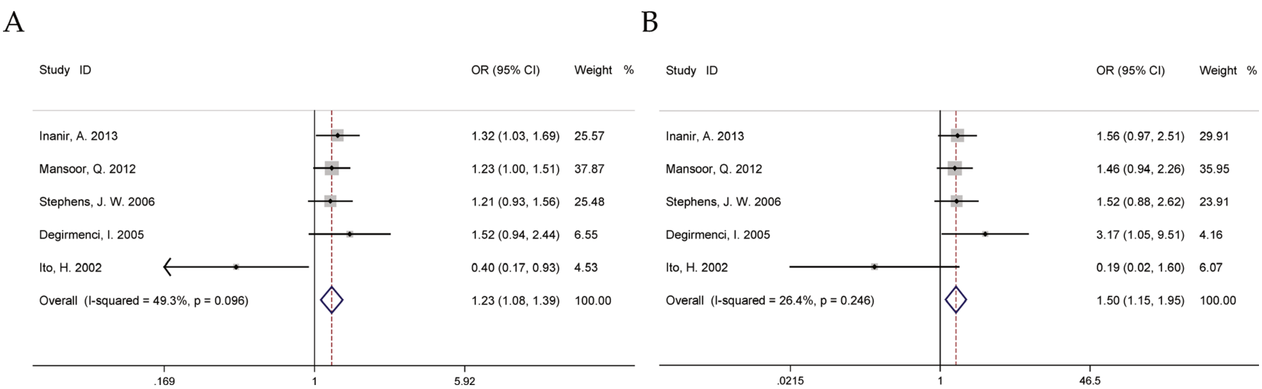

Figure 2. Forests for ACE I>D polymorphism and DN risk. (A) allele model (D vs. I); (B) homozygous model (DD vs. II). DN, diabetic neuropathy.

Table 2. Summary ORs and 95% CIs of ACE I>D polymorphism and DN risk.

		Allele (D vs. I)			Recessive (DD vs. ID + II)			Dominant (ID + DD vs. II)		
Locus	*N**	OR (95% CI)	*P*	I^2 (%)	OR (95% CI)	*P*	I^2 (%)	OR (95% CI)	*P*	I^2 (%)
Total	6	1.23 (1.08–1.39)	0.002	49.3	1.17 (0.97–1.41)	0.101	0	1.40 (0.91–2.14)	0.126	65.0
Ethnicity										
Asian	4	1.18 (0.89–1.58)	0.252	61.8	1.16 (0.91–1.46)	0.229	37.7	1.36 (0.76–2.44)	0.301	73.6
Caucasian	2	1.21 (0.93–1.56)	0.152	–	1.19 (0.88–1.62)	0.260	0	1.43 (0.87–2.33)	0.157	–
Quality of studies										
High-quality studies	4	1.06 (0.72–1.56)	0.764	71.8	1.27 (1.01–1.59)	0.043	0	1.03 (0.57–1.87)	0.919	66.5
Matched status										
Age and gender matched	4	1.06 (0.72–1.56)	0.764	71.8	1.27 (1.01–1.59)	0.043	0	1.03 (0.57–1.87)	0.919	66.5
Type of diabetes										
T2DM	4	1.15 (0.87–1.53)	0.333	60.1	1.05 (0.82–1.34)	0.688	0	1.40 (0.77–2.55)	0.268	72.4
T1DM	1	–		–	1.16 (0.69–1.95)	0.569	–	–		–
Type of neuropathy										
Sensorimotor neuropathy	2	0.75 (0.25–2.20)	0.596	83.5	1.14 (0.84–1.53)	0.404	0	0.77 (0.20–2.99)	0.700	82.5

ORs, odds ratios; CIs, confidence intervals; DN, diabetic neuropathy; T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus.
*Numbers of comparisons.

Association between genetic polymorphisms and DN risk

ACE I>D

The ACE I>D polymorphism was investigated in six studies along with DN (884 cases, 1570 controls).[17–22] A significant association was uncovered between the ACE I>D genetic polymorphism and DN risk under allelic and homozygous models (D vs. I: OR = 1.23, 95% CI = 1.08–1.39; DD vs. II: OR = 1.50, 95% CI = 1.15–1.95) (Fig. 2). Furthermore, stratified analyses based on ethnicity, quality of studies, matched status, type of diabetes and type of neuropathy were conducted for allele, recessive, and dominant models, with results presented in Table 2. Finally, increased susceptibility was found in the recessive model in the high-quality study group as well as in the age- and gender-matched group. We subsequently performed sensitivity analyses to explore the influence of an individual study on the pooled results, and our results did not

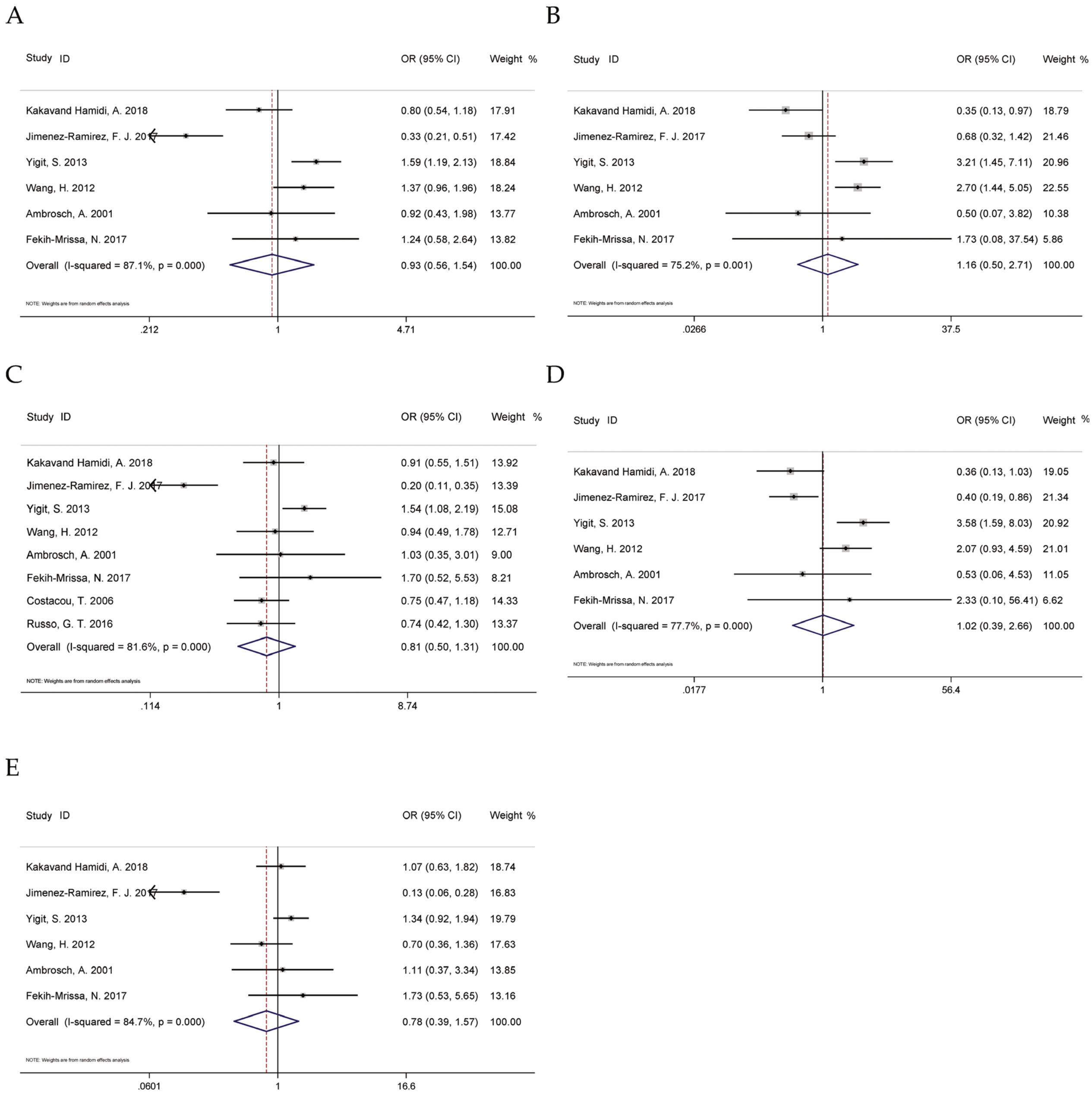

Figure 3. Forests for MTHFR C677T polymorphism and DN risk. (A) allele model (T vs. C); (B) recessive model (TT vs. TC + CC); (C) dominant model (TC + TT vs. CC); (D) homozygous model (TT vs. CC); (E) heterozygous model (TC vs. CC). DN, diabetic neuropathy.

change when omitting each study in the allelic and homozygous models (Figure S1).

MTHFR C677T and 1298A/C

Totally, there were 8 studies[13,14,20,23–27] (813 cases, 1544 controls) associated with MTHFR C677T and DN involved in the meta-analysis. Five of eight studies were performed in the Caucasian population, and the other three studies were performed in the Asian population. The pooled results of the five genetic models did not show any significant difference (Fig. 3). Further subgroup analyses were conducted, and no significant result was observed (Table 3).

We included three studies (223 cases, 650 controls) published on the relationship between the MTHFR 1298A/C polymorphism and DN in this meta-analysis.[23–25] Two of them were performed in the Caucasian population and the other one in the Asian population. Using the AA genotype as the reference, two genetic models revealed a significant association between the MTHFR 1298A/C polymorphism and DN (CC + AC vs. AA: OR = 1.44, 95% CI = 1.03–2.01; AC vs. AA: OR = 1.51, 95% CI = 1.07–2.11; Fig. 4). In addition, the stratified analyses according to ethnicity suggested that MTHFR 1298C/T was correlated with DN in the Caucasian population (CC + AC vs. AA: OR = 1.57, 95% CI = 1.02–2.41).

GPx-1 rs1050450

Five studies[31–33] (784 cases, 1957 controls) were combined to analyze the association between the GPx-1 rs1050450 polymorphism and DN. Three of five studies were performed in the Caucasian population, and the other two studies were conducted in the Asian population. The pooled OR values of four models revealed a significant association between GPx-1

Table 3. Summary ORs and 95% CIs of MTHFR C677T polymorphism and DN risk.

		Allele (T vs. C)			Recessive (TT vs. TC + CC)			Dominant (TC + TT vs. CC)		
Locus	*N**	OR (95% CI)	*P*	I^2 (%)	OR (95% CI)	*P*	I^2 (%)	OR (95% CI)	*P*	I^2 (%)
Total	8	0.93 (0.56–1.54)	0.784	87.1	1.16 (0.50–2.71)	0.732	75.2	0.81 (0.50–1.31)	0.396	81.6
Ethnicity										
Asian	3	1.22 (0.82–1.81)	0.321	74.5	1.53 (0.46–5.10)	0.489	85.2	1.22 (0.94–1.59)	0.135	43.3
Caucasian	5	0.69 (0.28–1.68)	0.416	82.5	0.68 (0.34–1.35)	0.272	0	0.66 (0.33–1.30)	0.227	79.8
Genotyping method										
PCR-RFLP	5	0.89 (0.50–1.57)	0.686	89.6	1.13 (0.46–2.78)	0.799	80.2	0.77 (0.36–1.64)	0.494	88.9
Others	3	1.24 (0.58–2.64)	0.580	–	1.73 (0.08–37.54)	0.728	–	0.80 (0.57–1.12)	0.200	0
Type of diabetes										
T2DM	6	0.82 (0.47–1.44)	0.497	84.4	0.88 (0.34–2.33)	0.803	73.4	0.73 (0.40–1.33)	0.299	78.5
T1DM	1	–		–	–		–	0.75 (0.47–1.18)	0.207	–
Type of neuropathy										
Sensorimotor neuropathy	4	1.14 (0.58–2.24)	0.701	86.9	1.09 (0.13–9.48)	0.939	91.1	0.97 (0.66–1.42)	0.864	64.0
Controls in HWE	3	1.09 (0.65–1.83)	0.742	75.6	0.90 (0.17–4.71)	0.895	83.6	1.27 (0.96–1.68)	0.092	30.7

ORs, odds ratios; CIs, confidence intervals; DN, diabetic neuropathy; PCR-RFLP, polymerase chain reaction and restriction fragment length polymorphism; T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; HWE, Hardy–Weinberg Equilibrium.
*Numbers of comparisons.

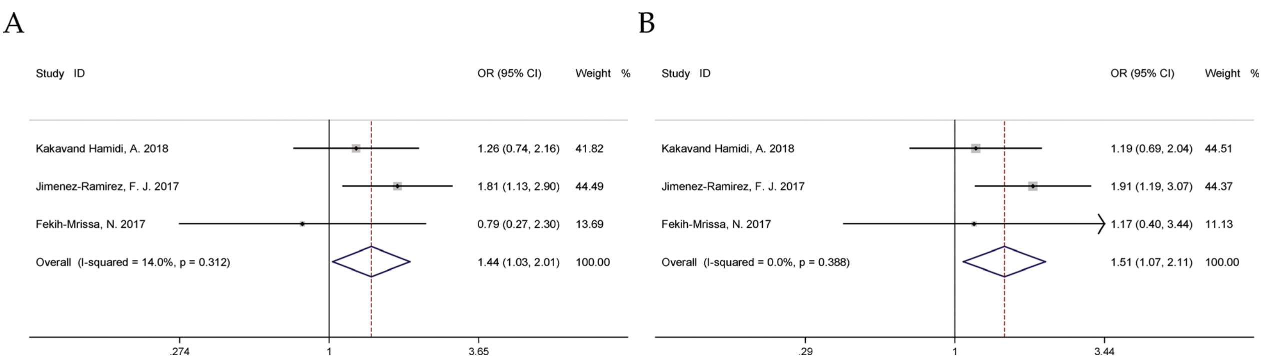

Figure 4. Forests for MTHFR 1298A/C polymorphism and DN risk. (A) dominant model (CC + AC vs. AA); (B) heterozygous model (AC vs. AA). DN, diabetic neuropathy.

rs1050450 and DN risk (T vs. C: OR = 1.43, 95% CI = 1.26–1.64; TT + CT vs. CC: OR = 1.74, 95% CI = 1.46–2.08; TT vs. CC: OR = 1.58, 95% CI = 1.17–2.12; CT vs. CC: OR = 1.78, 95% CI = 1.48–2.14; Fig. 5). Stratification accounting for the type of diabetes revealed increased DN risk in the T2DM group (Table 4). Additionally, a similar relationship was detected under allelic and dominant models in the group with Caucasian ethnicity, sensorimotor neuropathy and controls in HWE (Table 4). In addition, each single study was omitted sequentially, without obvious alteration of overall statistical significance in sensitivity analysis (Figure S1).

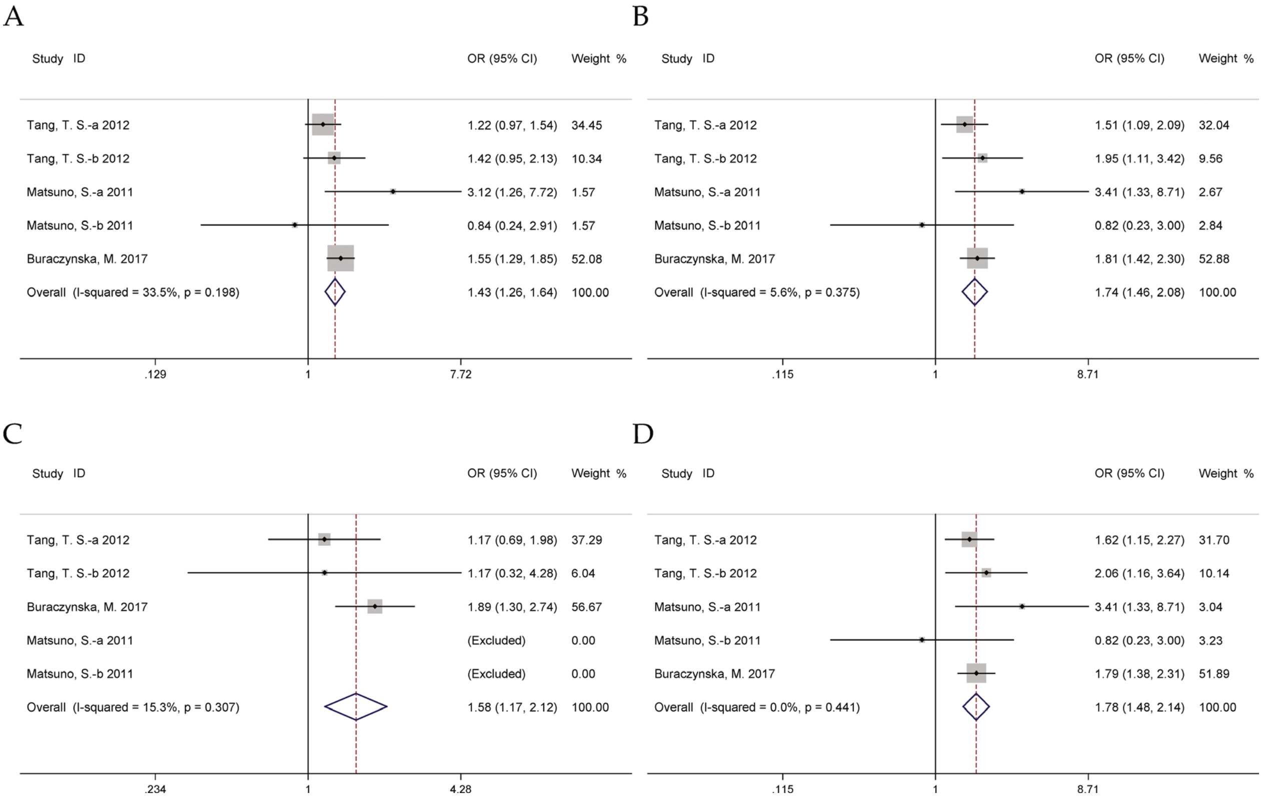

Figure 5. Forests for GPx-1 rs1050450 polymorphism and DN risk. (A) allele model (T vs. C); (B) dominant model (TT + CT vs. CC); (C) homozygous model (TT vs. CC); (D) heterozygous model (CT vs. CC). DN, diabetic neuropathy.

Table 4. Summary ORs and 95% CIs of GPx-1 rs1050450 polymorphism and DN risk.

		Allele (T vs. C)			Recessive (TT vs. CT + CC)			Dominant (TT + CT vs. CC)		
Locus	*N**	OR (95% CI)	*P*	I^2 (%)	OR (95% CI)	*P*	I^2 (%)	OR (95% CI)	*P*	I^2 (%)
Total	5	1.43 (1.26–1.64)	0.000	33.5	1.21 (0.92–1.59)	0.182	29.2	1.74 (1.46–2.08)	0.000	5.6
Ethnicity										
Caucasian	3	1.42 (1.24–1.62)	0.000	18.4	1.21 (0.92–1.59)	0.182	29.2	1.72 (1.44–2.07)	0.000	0
Asian	2	1.74 (0.48–6.26)	0.399	64.2	Excluded		–	1.80 (0.45–7.19)	0.404	67.0
Type of diabetes										
T2DM	3	1.57 (1.32–1.87)	0.000	37.6	1.46 (1.03–2.07)	0.035	–	1.84 (1.46–2.30)	0.000	36.6
Type of neuropathy										
Sensorimotor neuropathy	3	1.33 (1.09–1.62)	0.005	49.9	0.89 (0.56–1.41)	0.624	0	1.72 (1.32–2.25)	0.000	29.5
Autonomic neuropathy	1	0.84 (0.24–2.91)	0.778	–	Excluded		–	0.83 (0.23–3.00)	0.770	–
Both	1	1.55 (1.29–1.85)	0.000	–	1.46 (1.03–2.07)	0.035	–	1.81 (1.42–2.30)	0.000	–
Controls in HWE	3	1.55 (1.10–2.19)	0.013	41.1	0.79 (0.23–2.75)	0.711	–	1.99 (1.29–3.09)	0.002	34.5

ORs, odds ratios; CIs, confidence intervals; DN, diabetic neuropathy; PCR-RFLP, polymerase chain reaction and restriction fragment length polymorphism; T2DM, type 2 diabetes mellitus; HWE, Hardy–Weinberg Equilibrium.
*Numbers of comparisons.

CAT-262C/T

The analysis of the CAT -262C/T polymorphism associated with DN included 3 studies (350 cases, 465 controls), which were all performed in the Caucasian population.[28–30] Using the CC genotype as a reference, we found a protective effect of the CAT -262C/T polymorphism against the susceptibility of DN (T vs. C: OR = 0.71, 95% CI = 0.57–0.87; TT vs. CT + CC: OR = 0.53, 95% CI = 0.36–0.77; TT vs. CC: OR = 0.54, 95% CI = 0.35–0.82; Fig. 6). When stratified by the type of diabetes, a decreased risk was identified in the T1DM group (T vs. C: OR = 0.68, 95% CI = 0.53–0.86; TT vs. CT + CC: OR = 0.51, 95% CI = 0.35–0.76), but not T2DM group.

GSTM1 and GSTT1 null/present

The meta-analysis including four studies[30,34–36] (516 cases, 573 controls) about GSTM1 null/present polymorphism and DN reflected no significant difference (OR = 1.21, 95% CI = 0.94–1.56, Fig. 7). Concerning GSTT1 null/present polymorphism, four studies[30,34–36] (500 cases, 589 controls) were enrolled in the meta-analysis. The pooled results also failed to show any significant difference (OR = 0.96, 95% CI = 0.30–3.04, Fig. 8). The sensitivity analysis showed no significance after excluding any of the studies (Figure S1).

IL-10 -1082G/A

In the meta-analysis of the IL-10 -1082G/A polymorphism and DN, three studies[37–39] were involved (444 cases, 358 controls). The pooled results showed no significance between IL-10 -1082G/A and DN (Fig. 9).

Other genetic polymorphisms associated with DN

In addition to the genetic polymorphisms discussed above, we also found that some other polymorphisms had statistical significance on DN risk in 33 individual studies, such as CACNA 1A rs2248069, CYBA rs4673, FTO rs17817449, IL2RA rs706778, SCN10A rs7375036, CTLA-4 rs5742909, GNB3 C825T, and NOS3 Glu298Asp.[8,20,28,39–68] Due to the small number of relevant studies or insufficient data for genotype frequency, these studies could not be enrolled in our meta-analysis. Therefore, we performed a systematic review of these polymorphisms and listed them in Table 5, with the purpose of providing clues in future searches for genetic risk factors of DN.

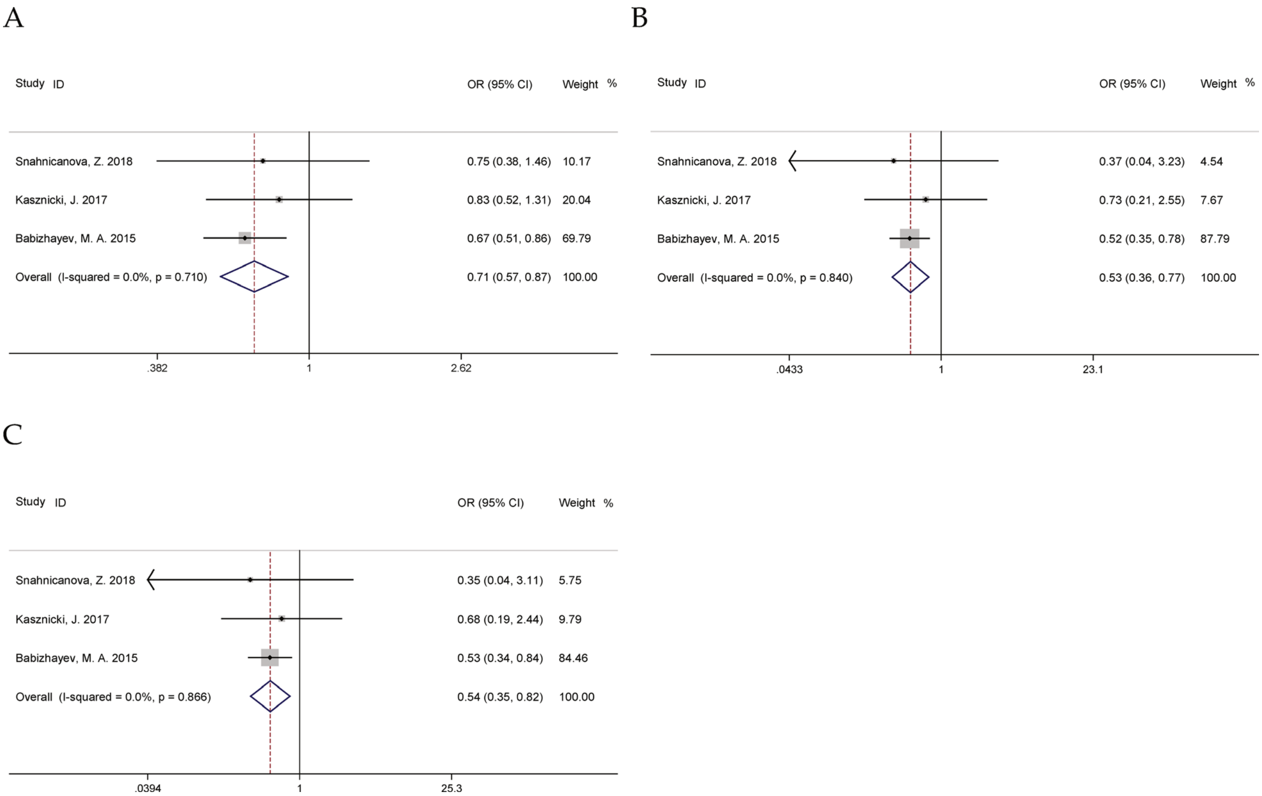

Figure 6. Forests for CAT-262C/T polymorphism and DN risk. (A) allele model (T vs. C); (B) recessive model (TT vs. CT + CC); (C) homozygous model (TT vs. CC). DN, diabetic neuropathy.

Detection of publication bias

Funnel plot and Egger's test were employed to appraise the publication bias among all eight studies. By visual detection of funnel plots, six genetic variants including ACE I>D, MTHFR C677T, GPx-1 rs1050450, CAT -262C/T, GSTM1 null/present and GSTT1 null/present, showed symmetric shapes, which demonstrated that no publication bias existed and was further confirmed by Egger's test. In contrast with these variants, we detected mild publication bias in MTHFR 1298A/C and IL-10 polymorphisms. As for MTHFR 1298A/C, marginal bias could be found in the allelic model (P = 0.025). In the recessive genetic model of IL-10, a statistically significant difference could be found by Egger's test (P = 0.023). The visual inspection of the funnel plot and P value of Egger's test of all included studies are summarized in Figure S2 and Table 6, respectively.

Trial sequential analysis

Among the eight studies mentioned above, three studies performed on the ACE I>D polymorphism, GPx-1 rs1050450 polymorphism, and IL-10 -1082G/A polymorphism concluded that a sufficient number of samples were used in the analyses, and conclusive results could be

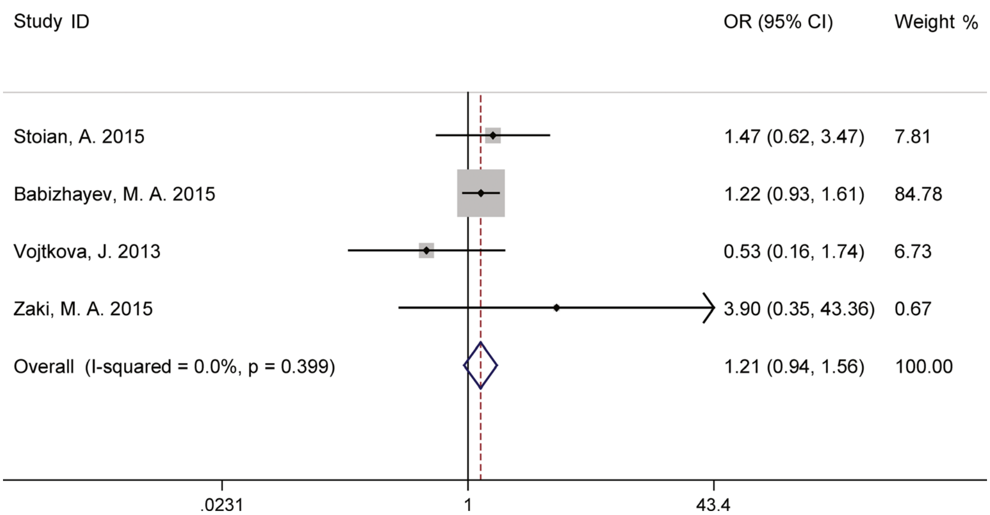

Figure 7. Forest for GSTM1 null/present polymorphism and DN risk. DN, diabetic neuropathy.

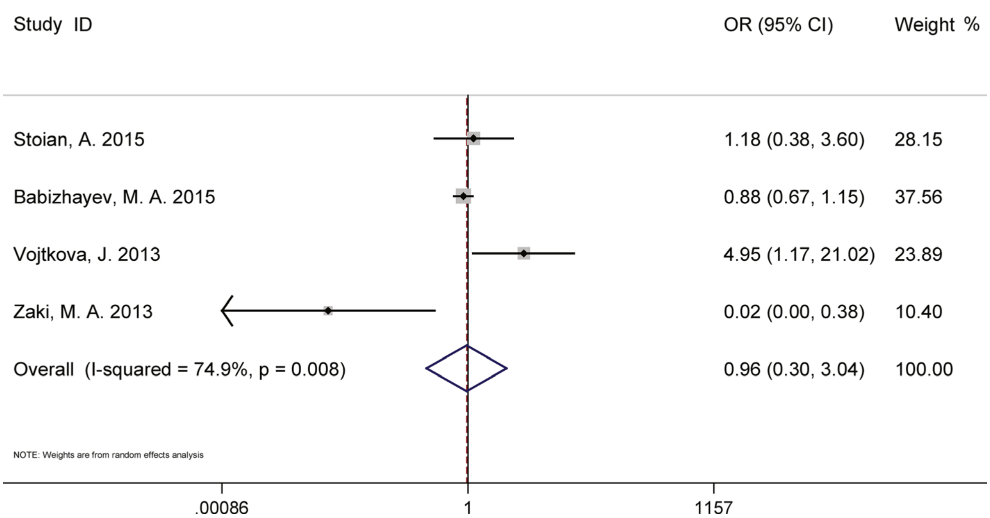

Figure 8. Forest for GSTT1 null/present polymorphism and DN risk. DN, diabetic neuropathy.

obtained. Specifically, in the study of the ACE I>D polymorphism, the *Z*-curve of the allelic and homozygous model crossed either the TSA monitoring boundary or RIS line, confirming that the ACE I>D polymorphism was associated with increased DN risk. For the GPx-1 rs1050450 polymorphism, in the allelic, dominant and heterozygous models, we detected that the *Z*-curve exceeded the RIS line, which revealed enough evidence for significant results. With regard to the IL-10 -1082G/A polymorphism, as the *Z*-curve entered the futility area in the allelic and dominant models, we came to a confirmed conclusion that the IL-10 polymorphism had no relationship with DN susceptibility. However, the TSA results of the other five genetic variants did not show adequate information involved in the meta-analysis. More relevant studies are necessary to prove our findings in the future. The TSA results for all the included studies are shown in Figure S3.

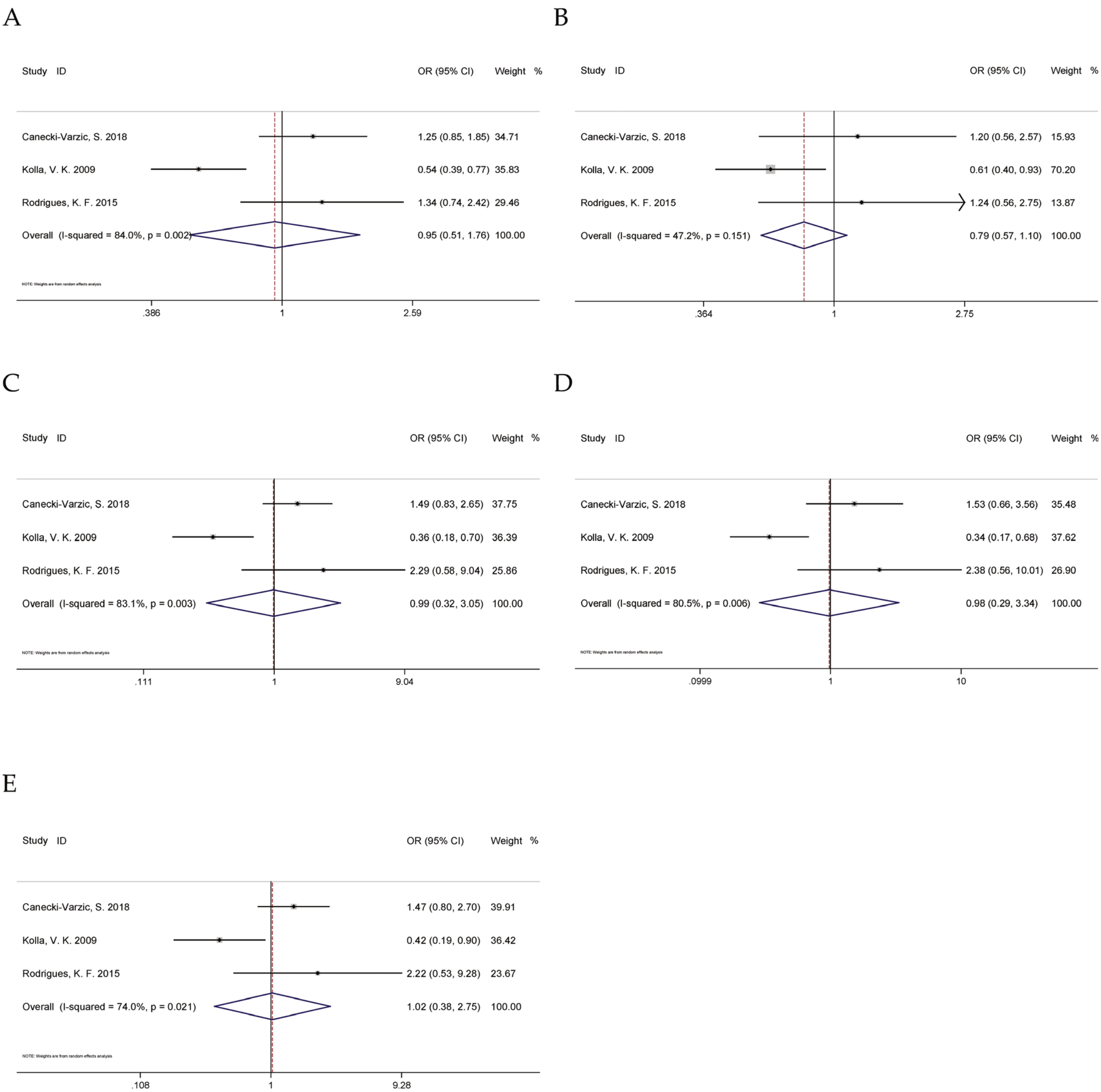

Figure 9. Forests for IL-10 -1082G/A polymorphism and DN risk. (A) allele model (G vs. A); (B) recessive model (AA vs. AG + GG); (C) dominant model (AA + AG vs. GG); (D) homozygous model (AA vs. GG); (E) heterozygous model (AG vs. GG). DN, diabetic neuropathy.

Table 5. Systematic review for polymorphisms not enrolled into our meta-analysis.

Author	Year	Ethnicity	Gene/variant	Comparison	No. of cases/controls	OR (95% CI)	*P*-value	NOS	References
Sun, L.	2018	Chinese	CACNA 1A/rs2248069	A versus G	143/180	8.27 (3.93–17.40)	<0.001	6	40
Sun, L.	2018	Chinese	CACNA 1A/rs16030	C versus T	143/180	6.25 (2.86–13.67)	<0.001	6	40
Sun, L.	2018	Chinese	CACNA 1C/rs216008	C versus T	143/180	2.58 (1.45–1.60)	0.001	6	40
Sun, L.	2018	Chinese	CACNA 1C/rs2239050	G versus C	143/180	6.01 (2.59, 13.94)	<0.001	6	40
Sun, L.	2018	Chinese	CACNA 1H/rs3794619	C versus T	143/180	2.52 (1.52, 4.17)	<0.001	6	40
Sun, L.	2018	Chinese	CACNA 1H/rs7191246	G versus C	143/180	7.38 (3.11, 17.56)	<0.001	6	40
Snahnicanova, Z.	2018	Slovak	CYBA/rs4673	C versus T	34/80	5.00 (1.40–19.08)	0.016	6	28
Hubacek, J. A.	2018	Czech	FTO/rs17817449	G versus T	474/339	1.59 (1.11–2.29)	0.005	6	41
Zaky, E. A.	2018	Egyptian	IL2RA/rs706778	A versus G	200/200	13.63 (7.52–24.71)	<0.001	7	42
Ciccacci, C.	2018	Italy	MIR499A/rs3746444	A versus G	69/80	1.92 (1.00–3.70)	0.005	7	43
Ezhilarasi, K.	2018	Indian	VDR/rs1544410	A versus G	72/–	9.86 (4.88–19.91)	0.001	6	44
Marzban, A.	2017	Iranian	HLA-DQB1/DQB1*02 allele	DQB1*02 allele	49/57	–	–	7	45
Marzban, A.	2017	Iranian	HLA-DRB1/DRB1*10/DRB1*12 alleles	DRB1*10/DRB1*12 alleles	49/57	–	–	7	45
Gupta, B.	2017	Indian	AR/rs759853	C versus T	356/294	1.97 (1.16–3.35)	0.015	7	46
Lv, Y.	2017	Chinese	SCN10A/rs7375036	C versus T	–	–	–	7	47
Kiani, J.	2016	Iranian	CTLA-4/rs5742909	C versus T	49/100	2.56 (1.31–4.98)	0.006	6	48
Ji, Z. Y.	2015	Chinese	ADP/rs1501299	G versus T	90/90	2.69 (1.54–4.67)	<0.001	8	49
Chen, Y.	2015	Chinese	ADP/rs3774261	A versus G	80/80	3.18 (1.77–5.72)	<0.001	7	50
Chen, Y.	2015	Chinese	ADP/rs3821799	T versus C	80/80	2.31 (1.30–4.08)	0.004	7	50
Ren, Z.	2015	Chinese	ICAM-1/rs1799969	A versus G	399/383	3.70 (1.21–11.28)	0.014	7	51
Ren, Z.	2015	Chinese	ICAM-1/rs281432	C versus G	399/383	1.20 (1.01–1.43)	0.041	7	51
Ren, Z.	2015	Chinese	ICAM-1/rs5498	A versus G	399/383	1.72 (1.03–2.87)	0.037	7	51
Jia, Y.	2015	Chinese	GRP78/rs391957	C versus T	97/198	2.23 (1.42–3.52)	0.001	7	52
Ciccacci, C.	2014	Italy	MIR128a/rs11888095	C versus T	61/64	2.91 (1.32–6.44)	0.007	6	53
Ciccacci, C.	2014	Italy	MIR146a/rs2910164	G versus C	27/100	0.46 (0.22–0.94)	0.032	6	53
Ciccacci, C.	2014	Italy	MIR27a/rs895819	A versus G	26/97	3.20 (1.30–7.78)	0.009	6	53
Zhang, X.	2014	Chinese	VEGF/C936 T	C versus T	204/184	–	–	7	54
Groener, J. B.	2013	Germany	Glo1/rs4746	C versus A	251/273	–	–	7	55
Basol, N.	2013	Turkish	IL-4/VNTR(P1)	P1 versus P2	227/241	2.28 (1.46–3.58)	<0.001	7	56
Ciccacci, C.	2013	Italian	TCF7L2/rs7903146	C versus T	13/49	3.88 (1.53–9.81)	0.015	7	57
Korzon-Burakowska, A.	2012	Poland	OPG/rs3102734	C versus T	44/95	–	–	6	58
Korzon-Burakowska, A.	2012	Poland	OPG/rs2073617	T versus C	44/95	–	–	6	58
Korzon-Burakowska, A.	2012	Poland	OPG/rs3134069	T versus G	44/95	–	–	6	58
Mehrab-Mohseni, M.	2011	Iranian	NOS3/intron 4 VNTR	a versus b	146/96	1.80 (1.00–3.70)	0.03	6	59
Tavakkoly-Bazzaz, J.	2010	Iranian	VEGF/-7 C/T	C versus T	82/166	1.91 (1.03–3.60)	0.020	7	60
Kolla, V. K.	2009	Indian	IFN-γ/+874A/T	A versus T	198/202	1.40 (1.06–1.90)	0.012	7	39
Chistiakov, D. A.	2009	Russian	GNB3/C825T	C versus T	100/113	2.44 (1.60–3.73)	<0.001	6	61
Yang, L.	2008	Chinese	MT1B/rs11076161	A versus G	–	–	–	6	62
Yang, L.	2008	Chinese	MT2A/rs10636	G versus C	–	–	–	6	62
Nikitin, A. G.	2008	Russian	PARP-1/Leu54Phe	Leu/Phe	93/86	1.66 (1.08–2.54)	0.023	6	63

(*Continued*)

Table 5. Continued.

Author	Year	Ethnicity	Gene/variant	Comparison	No. of cases/controls	OR (95% CI)	*P*-value	NOS	References
Nikitin, A. G.	2008	Russian	PARP-1/Val762Ala	Val/Ala	93/86	2.88 (1.43–5.77)	0.002	6	63
Papanas, N.	2007	Greek	Alpha2B-AR/I/D	I versus D	130/60	–	0.001	7	64
Costacou, T.	2006	USA	NOS3/Glu 298 Asp	G versus T	114/256	4.86 (1.04–22.72)	<0.05	7	20
Rudofsky, G., Jr.	2006	Germany	UCP2/G-866A	G versus A	–	0.44 (0.24–0.79)	0.007	5	65
Rudofsky, G., Jr.	2006	Germany	UCP3/C-55T	C versus T	–	0.48 (0.25–0.92)	0.031	5	65
Rudofsky, G., Jr.	2004	Germany	TLR4/Asp299Gly + Thr399Ile	Asp versus Gly Thr versus Ile	–	–	–	7	66
Benjafield, A. V.	2001	Australia	TNFRSF1B/CA16 allele	I versus D	69/230	2.10 (1.20–3.80)	–	6	67
Shi, H.	1998	Chinese	ApoA/S2/S3/S4	S2 versus S3 versus S4	26/150	–	–	6	68
Vague, P	1997	Caucasian	ATP1A1/restricted allele	I versus D	31/50	–	–	6	8

ORs, odds ratios; CIs, confidence intervals; NOS, Newcastle-Ottawa Quality Assessment Scale.

Discussion

As we all know, the systematic review and meta-analysis approach used in this study is the most comprehensive method to detect genetic risk factors in most human diseases.[69] To date, there is no complete systematic review and meta-analysis reporting the potential association between all genetic polymorphisms and DN risk. Using widely accepted genetic models and subgroup analyses based on ethnicity, HWE status, quality of studies and so on, we performed this comprehensive systematic review that provided empirical support for exploring the relationship between relevant genetic polymorphisms, such as ACE I/D, MTHFR C677T, MTHFR 1298 A/C, GPx-1 rs1050450, CAT -262C/T, GSTM1, GSTT1, IL-10 -1082G/A, and DN susceptibility.

ACE is a key component of the renin–angiotensin system that converts angiotensin (Ang) I to Ang II. Ang II impacts endothelial damage and microcirculatory dysfunction.[70] Therefore, insufficient blood supply to peripheral nerves due to microcirculatory dysfunction is considered a possible pathological mechanism of DN.[71] As the starting factor affecting Ang II level, ACE activity is influenced by the presence of an insertion (I) or deletion (D) of a 287-base pair fragment in intron 16 of the ACE gene resulting in a common variant, with the D allele being associated with higher ACE activity.[72] This allele has been previously observed to probably associate with microvascular complications of diabetes.[73–75] In this study, we statistically confirmed that the ACE I/D polymorphism was significantly associated with DN risk. The D allele had a 1.23-fold risk for DN compared with the I allele, and a 50% increased risk of DN was identified in DN patients with the DD genotype compared with the II genotype.

MTHFR is a key regulatory enzyme in homocysteine metabolism that converts homocysteine back to methionine via the re-methylation pathway.[76] Therefore, deficiency of MTHFR increases the odds for hyperhomocysteinemia.[77] Meantime, it was reported that homocysteine levels and the prevalence of hyperhomocysteinemia were strongly associated with DN.[78] Mutations of the MTHFR gene have been defined, and C677T and A1298C variants are the two of the most explored.[77] Both are functional polymorphisms that lead to decreased enzymatic activity, resulting in elevated homocysteine levels.[77] The association between MTHFR gene polymorphisms and the susceptibility of DN has been investigated in several studies but with inconsistent results. Therefore, we performed this meta-analysis involving all the available evidence of these two genetic variants and DN risk. In our study, only the MTHFR 1298A/C polymorphism showed a significant association with DN in the pooled

Table 6. Summary of *P* values of Egger's test for various contrasts of genetic polymorphisms and diabetic neuropathy susceptibility.

Polymorphism	Allelic model	Recessive model	Dominant model	Homozygous model	Heterozygous model
ACE I/D	0.293	0.279	0.579	0.581	0.609
MTHFR C677T	0.512	0.383	0.682	0.712	0.514
MTHFR 1298A/C	0.025	–	0.329	–	0.655
GPx-1	0.880	0.510	0.933	0.577	0.880
CAT-262C/T	0.460	0.925	0.669	0.913	0.469
GSTM1 null/present	0.957	–	–	–	–
GSTT1 null/present	0.349	–	–	–	–
IL-10	0.535	0.023	0.866	0.441	0.936

results, while no significant difference was found in the analysis of MTHFR C677T. In vitro studies showed that hyperhomocysteinemia affected nervous function either by direct cytotoxicity or by oxidative damage.[79,80] Oxidative stress is associated with the development of apoptosis in neurons and supporting glial cells and could be the unifying mechanism that leads to nervous system damage in diabetes.[81,82]

GPx-1 is a gene that encodes an antioxidant enzyme. Its main role is protecting cells against oxidative damage by reducing hydrogen peroxide and organic peroxidases to H_2O_2 with reduced glutathione.[83] As one of the GPx-1 polymorphisms, rs1050450, which reduces the activity of this enzyme, may cause an adverse effect on the vascular system and microvascular complications of diabetes.[84,85] The present study aimed to evaluate the association of the rs1050450 polymorphism in the GPx-1 gene with DN. For our pooled results, we detected that GPx-1 rs1050450 showed a significant difference in the risk for DN. In the subgroup analysis, we found a similar result in the Caucasian population, as well as in the T2DM and sensorimotor neuropathy groups. The exact mechanism of the observed effect of GPx-1 gene polymorphism on susceptibility to DN is unknown. We speculate that changing the capacity of the antioxidant enzyme by the rs1050450 polymorphism may lead to increased oxidative damage which was found to be an important pathophysiological mechanism involved in DN.

CAT is a widespread enzyme that can catalyze the decomposition of H_2O_2 to water and molecular oxygen, which inactivate free oxygen radicals and peroxides in the process of oxidative stress existing in DN.[86] Therefore, CAT plays an important role in the pathogenesis of DN. From the current meta-analysis of CAT -262C/T and DN risk, our findings suggested that the T allele showed a protective effect on DN development, with nearly 29% and 47% decreased susceptibility in the allelic and recessive genetic models, respectively. Additionally, all three studies involved in this meta-analysis are performed in the Caucasian population. Thus, there may be a low risk for DN in T allele carriers of Caucasians. However, no related study was conducted in an Asian population. The role of CAT -262C/T in DN requires further studies for non-Caucasian populations.

Glutathione S-transferases (GSTs) are a family of antioxidant enzymes that play important antioxidant roles in the elimination of reactive oxygen species.[87] GSTM1 and GSTT1 genes are polymorphic in humans, and the null genotypes are accompanied by a lack of enzyme activity.[88] The GSTM1 and GSTT1 polymorphisms have been reported as risk factors for DN in the past but without consistent results. According to our pooled data, none of these two genetic polymorphisms showed a significant difference in the risk for DN. However, due to the limited number of further studies and the inadequate number of included samples indicated in TSA, confirming the association between either of the two genetic polymorphisms and DN is difficult. Future studies with larger sample sizes are required.

Limitation also existed in our study. First, several genes have just been investigated in small cohorts and in only Caucasian populations such as GSTT1, GSTM1, and CAT -262C/T. Second, we confined the enrolled studies to publications in English. Third, obvious heterogeneity could be detected among some meta-analyses, such as MTHFR C677T and GSTM1 null/present which influences the credibility of our results. Therefore, we performed subgroup and sensitivity analyses to explore the source of heterogeneity, which was often from different study designs, measurement errors and ethnic diversity. Unfortunately, heterogeneity was not eliminated by these methods, which indicated that all factors mentioned before should be considered together. Fourth, mild publication bias was detected in MTHFR 1298A/C and IL-10 polymorphisms, and TSA showed inadequate information involved in the analyses for MTHFR, CAT and GST genes. Thus, the comprehensive analyses should be interpreted with caution. Finally, we did not analyze the gene-gene and gene-environment interactions in our current meta-analysis due to insufficient information.

In conclusion, we demonstrated that ACE I/D, MTHFR 1298A/C, GPx-1 rs1050450, and CAT-262C/T were associated with DN susceptibility but MTHFR C677T, GSTM1, GSTT1, and IL-10 −1082G/A were not. More studies performed in different ethnicities with larger sample sizes are required to confirm our findings in the near future.

Acknowledgments

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Conflict of Interest

The authors declare no financial or other conflicts of interests.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Sensitivity analyses for the polymorphisms and DN risk.
Figure S2. Funnel plots for the polymorphisms and DN risk.
Figure S3. Trial sequential analyses for the polymorphisms and DN risk.
Table S1. Full genetic polymorphism list for systematic review.

