

Original Article

The meta-analysis of CCR5 genetic variants and diabetic nephropathy risk

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Abstract: Background: Some studies reported the association between CCR5 rs333, rs1799987 and diabetic nephropathy (DN) risk. However, the results were controversial. Therefore, we performed this meta-analysis. Method: We searched PubMed and Embase databases. The strength of association was assessed by computing odds ratio (OR) with its corresponding 95% confidence interval (CI). Results: Nine studies with including 3475 cases and 3492 controls were included. The result showed that rs1799987 polymorphism significantly predicted DN risk (OR=1.38, 95% CI, 1.17-1.63). In the stratified analysis by ethnicity, the significant association was observed in Asians and Caucasians. In the subgroup analysis by type of diabetes, both T1D and T2D patients with rs1799987 polymorphism had increased DN risk. In addition, rs1799987 polymorphism significantly associated with EDN risk, while this polymorphism was not associated with AND risk. As for rs333, no significant association between this polymorphism and DN risk was found (OR=1.36, 95% CI, 0.79-2.33), even in the subgroup analyses. Conclusion: In conclusion, this meta-analysis suggests that individuals with CCR5 rs333 polymorphism may have an increased DN risk.

Keywords: Diabetic nephropathy, CCR5, association

Introduction

Diabetic nephropathy (DN) is a serious complication of diabetes mellitus and is a major cause of end-stage renal disease worldwide. DN is a multifactorial progressive disease with complex pathogenesis, involving hyperglycemia, advanced glycation end products, hemodynamic disorder, metabolic abnormalities, inflammatory factors, and genetic factors [1].

C-C chemokine receptor 5 (CCR5) is implicated in immune cell migration and cytokine release. The CCR5 gene is located on human chromosome 3p21.3-p24 within the chemokine receptor gene cluster and comprises three exons spanning a region of about 6 kb [2]. Several polymorphisms in this gene have been described. The deletion of 32 bp in exon 3 (rs333) changes the open reading frame of CCR5 and results in a nonfunctional truncated protein [3]. The 59029 G/A polymorphism (rs1799987) in the promoter region of the CCR5 gene causes the increased transcriptional activity of the CCR5 gene, increasing the protein level [4].

Recently, many studies reported the association between rs333, rs1799987 and DN risk. However, the results were controversial [5-13]. Therefore, we performed this meta-analysis to determine this association.

Methods

Search for publications

We searched the articles using the search terms "C-C chemokine receptor 5", "CCR5", "diabetic nephropathy" and "genetic" in the PubMed and Embase. The last search updated on Apr 2016. Additional studies were identified by a hand search of references of original studies or review articles. No publication date or language restriction were imposed.

Inclusion and exclusion criteria

The following inclusion criteria were used: (1) the study should have evaluated the association between rs333, rs1799987 and DN risk; (2) the study should have a case-control design; (3) sufficient data should have been provided in

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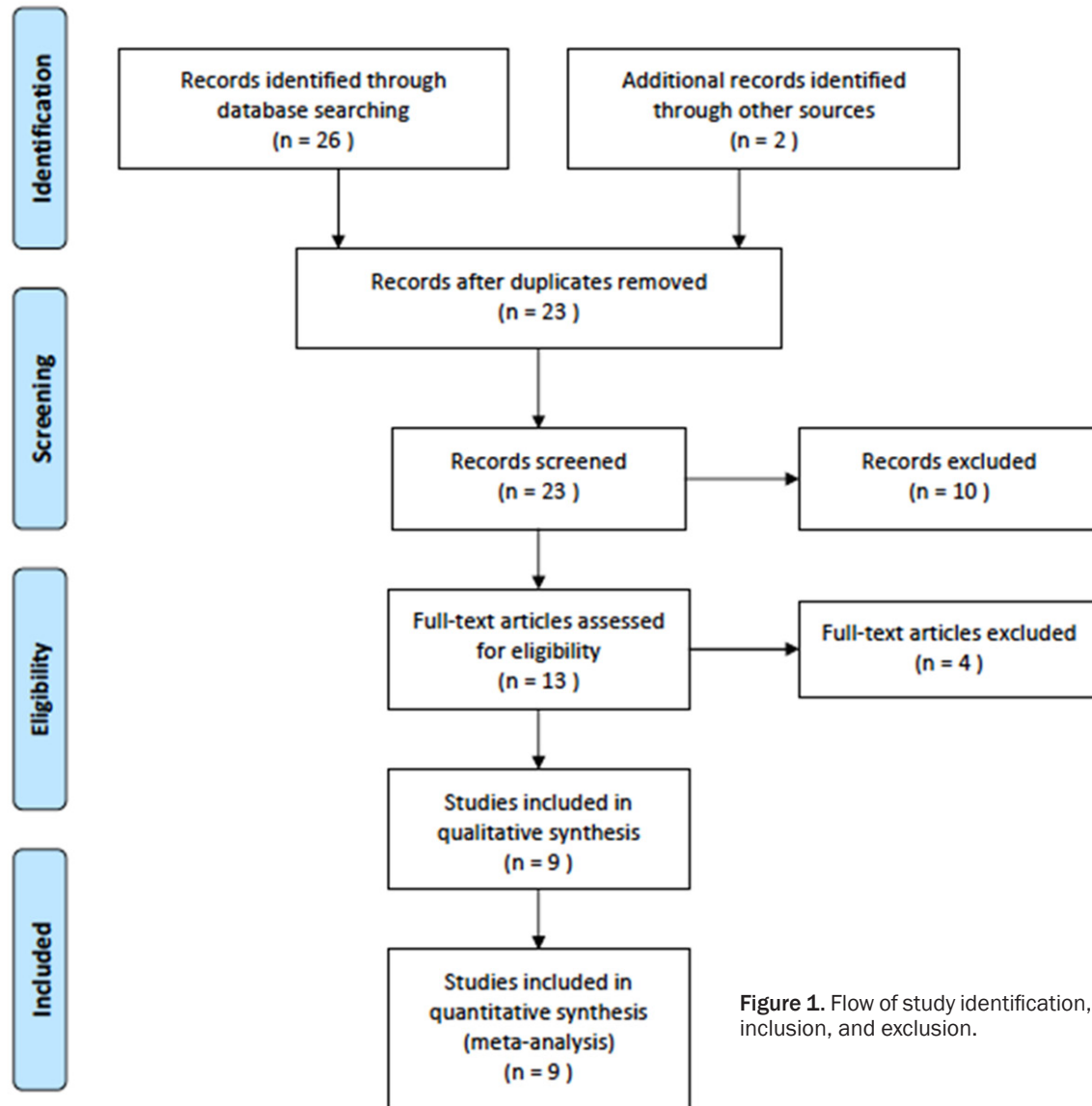


Figure 1. Flow of study identification, inclusion, and exclusion.

order to calculate odds ratios (OR) and 95% confidence intervals (CI). Studies were excluded if any of the following conditions applied: (1) irrelevant to CCR5 or DN; (2) abstract or review; (3) non-clinical study; (4) studies were repeated.

Data extraction

Two investigators independently extracted data and reached consensus on the following characteristics of the selected studies: the first author's name, year of publication, ethnicity of the study population, age, gender, type of diabetes, type of DN, numbers of cases and controls, CCR5 polymorphism, and Hardy-Weinberg equilibrium (HWE).

Quality assessment

The included studies were assessed independently by the two reviewers using the Newcastle-Ottawa Scale (NOS). The NOS employs a star rating system to assess quality from 3 broad perspectives of the study: (1) selection of the study groups, (2) comparability of the groups, and (3) identification of the exposure (for case-control studies) or outcome of interest (for cohort studies). Scores ranged from 0 to 9 stars.

Statistical analysis

OR and 95% CI were employed to evaluate the strength of the association between rs333,

Table 1. Characteristics of the included studies

First author	Year	Ethnicity	Age	Gender	Type of diabetes	Type of DN	Case (n)	Control (n)	Polymorphism	HWE	Quality
Nakajima	2003	Asian	60	Mixed	T1D	EDN	261	355	rs1799987	Yes	7
Mlynarski 1	2005	Caucasian	37	Male	T1D	ADN	275	136	rs1799987, rs333	Yes	8
Mlynarski 2	2005	Caucasian	38	Female	T1D	ADN	221	162	rs1799987, rs333	Yes	8
Mokubo	2006	Asian	60	Mixed	T2D	EDN	71	120	rs1799987	Yes	8
Prasad	2007	Asian	61	Mixed	T2D	ADN	225	196	rs1799987	Yes	7
Tregouet 1	2008	Caucasian	41.8	Mixed	T1D	EDN	489	463	rs1799987	Yes	9
Tregouet 2	2008	Caucasian	41.3	Mixed	T1D	EDN	387	469	rs1799987	Yes	9
Tregouet 3	2008	Caucasian	43.1	Mixed	T1D	EDN	300	391	rs1799987	Yes	9
Ahluwalia 1	2009	Asian	60.3	Mixed	T2D	EDN	240	255	rs1799987, rs333	Yes	7
Ahluwalia 2	2009	Asian	60.1	Mixed	T2D	EDN	96	92	rs1799987, rs333	Yes	7
Pettigrew	2010	Caucasian	NA	Mixed	T1D	EDN	267	442	rs1799987, rs333	Yes	7
Buraczynska	2012	Caucasian	56	Mixed	T2D	EDN	441	196	rs1799987	Yes	8
Yadav	2014	Asian	56.7	Mixed	T2D	EDN	202	215	rs1799987	Yes	9

EDN, Established diabetic nephropathy; ADN, Advanced diabetic nephropathy; HWE, Hardy-Weinberg equilibrium; NA, Not available.

rs1799987 and DN risk. Departure from HWE in controls was tested by the chi-square test. The Q statistic and the I^2 statistic were used to assess the degree of heterogeneity among the studies included in the meta-analysis. If heterogeneity was observed among the studies, the random-effects model was used to estimate the pooled OR (the DerSimonian and Laird method). Otherwise, the fixed-effects model was adopted (the Mantel-Haenszel method). Subgroup analyses were carried out to find the source of heterogeneity. The potential publication bias was examined visually in a funnel plot of log [OR] against its standard error (SE), and the degree of asymmetry was tested using Egger's test.

All statistical tests were performed using Revman 5.1 software (Nordic Cochrane Center, Copenhagen, Denmark) and STATA 11.0 software (Stata Corporation, College Station, TX, USA). A P value <0.05 was considered statistically significant, except for tests of heterogeneity where a level of 0.10 was used.

Results

Study characteristics

We retrieved 28 studies totally from PubMed and Embase databases. After reading these titles and abstracts, 15 articles were excluded because they were letters, reviews, meeting abstracts, duplicate studies, or other reasons. Of the remained 13 articles, their full-text were obtained. After detailed evaluation, 4 studies in-

cluding 2 articles lacking data and 2 articles lacking clinical variables were excluded. Finally, 9 eligible literatures (13 case-control studies) were enrolled in this meta-analysis (**Figure 1**). The main characteristics of 9 articles are summarized in **Table 1**.

Results of the meta-analysis

rs1799987 polymorphism and DN risk: We utilized 13 literatures to accomplish a meta-analysis that assessing the association between rs1799987 polymorphism and DN risk. As shown in **Figure 2**, the random-effects model was used to evaluate the pooled OR with corresponding 95% CI, because the considerable heterogeneity among the pooled studies ($I^2=78%$). The result showed that rs1799987 polymorphism significantly predicted DN risk (OR=1.38, 95% CI, 1.17-1.63). In the race subgroup analysis, both Caucasians (OR=1.20; 95% CI, 1.00-1.43) and Asians (OR=1.68; 95% CI, 1.33-2.11) with rs1799987 polymorphism had increased DN risk. In the subgroup analysis according to type of diabetes, both T1D (OR=1.10; 95% CI, 1.00-1.20) and T2D (OR=1.79; 95% CI, 1.58-2.02) with rs1799987 polymorphism had increased DN risk. In the subgroup analysis of type of DN, rs1799987 polymorphism significantly associated with established diabetic nephropathy (EDN) (OR=1.41; 95% CI, 1.16-1.71), while this polymorphism was not associated with advanced diabetic nephropathy (ADN) (OR=1.30; 95% CI, 0.90-1.90). Funnel plot was performed to

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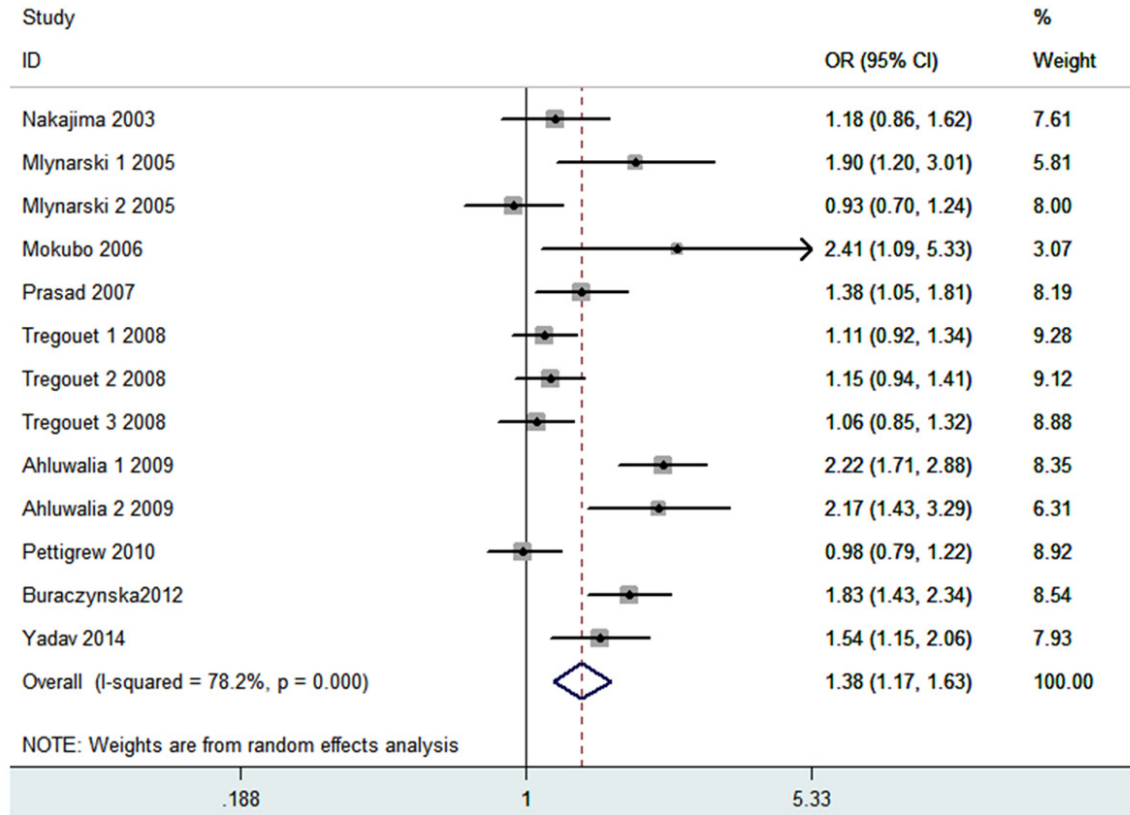


Figure 2. Meta-analysis for the association between rs1799987 polymorphism and DN risk.

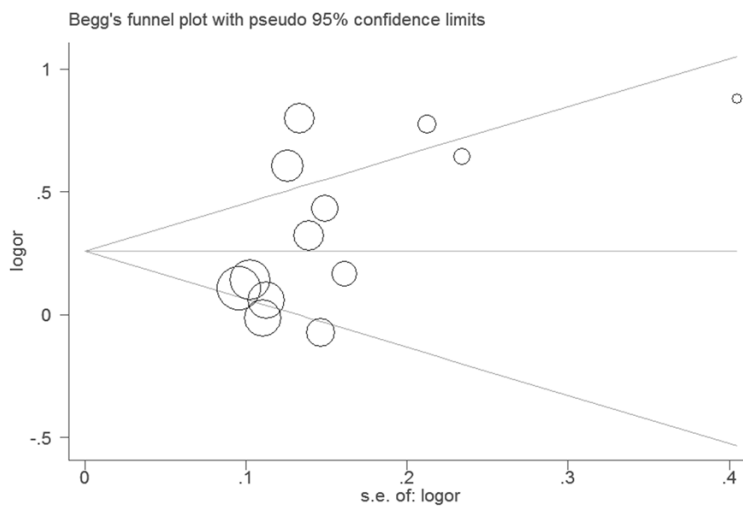


Figure 3. Funnel plot of the association between rs1799987 polymorphism and DN risk.

assess the publication bias of literatures. The shape of the funnel plot showed symmetry (**Figure 3**). Egger's test found no evidence of publication bias ($P=0.06$).

rs333 polymorphism and DN risk: We utilized 5 case-control studies to accomplish a meta-analysis that assessing the association between rs333 polymorphism and DN risk. As shown in **Figure 4**, the random-effects model was used to evaluate the pooled OR with corresponding 95% CI, because the considerable heterogeneity among the pooled studies ($I^2=88\%$). The result showed that rs333 polymorphism did not significantly predicted DN risk (OR=1.36, 95% CI, 0.79-2.33). In the race subgroup analysis, both Caucasians (OR=1.22; 95% CI, 0.66-2.28) and Asians (OR=1.55; 95% CI, 0.54-4.44) with rs333 polymorphism did not show increased DN risk. In the subgroup analysis according to type of diabetes, both T1D (OR=1.55; 95% CI, 0.54-

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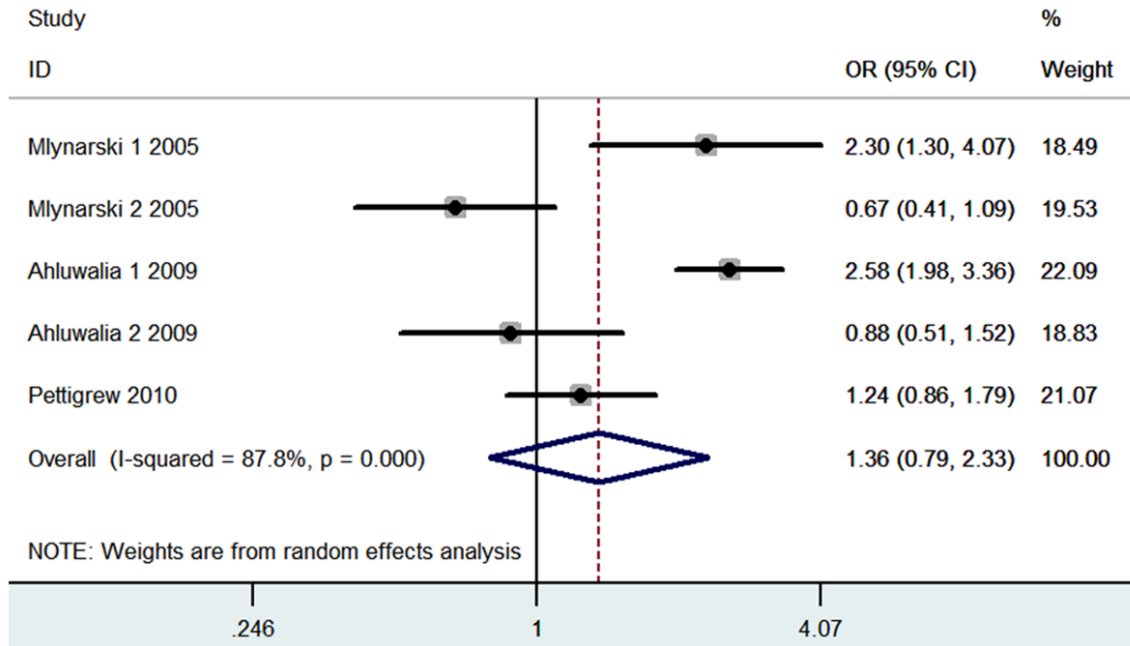


Figure 4. Meta-analysis for the association between rs333 polymorphism and DN risk.

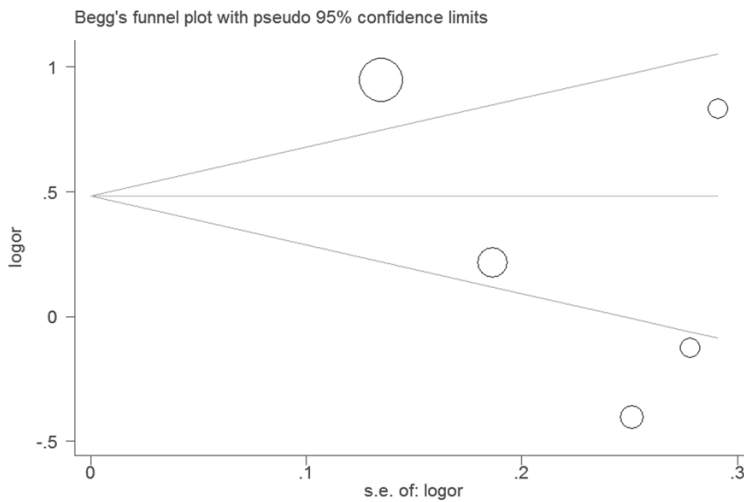


Figure 5. Funnel plot of the association between rs333 polymorphism and DN risk.

4.44) and T2D (OR=1.22; 95% CI, 0.66-2.28) with rs333 polymorphism did not have increased DN risk. In the subgroup analysis of type of DN, rs333 polymorphism did not significantly associated with EDN risk (OR=0.88; 95% CI, 0.52-1.47) and ADN risk (OR=1.46; 95% CI, 0.76-2.78). Funnel plot was performed to assess the publication bias of literatures. The shape of the funnel plot showed symmetry (**Figure 5**). Egger's test found no evidence of publication bias ($P=0.21$).

The results are listed in **Table 2**.

Discussion

In this meta-analysis, we investigated the association between CCR5 rs333, rs1799987 and DN risk including 3475 cases and 3492 controls. We found that individuals with rs1799987 polymorphism showed an increased risk of DN risk. In the stratified analysis by ethnicity, the significant association was observed in Asians and Caucasians. In the subgroup analysis by type of diabetes, both T1D and T2D patients

with rs1799987 polymorphism had increased DN risk. In addition, rs1799987 polymorphism significantly associated with EDN risk, while this polymorphism was not associated with ADN risk. As for rs333, no significant association between this polymorphism and DN risk was found, even in the subgroup analyses.

CCR5 rs1799987 polymorphism is a putatively functional variant in the promoter region, which affects the CCR5 protein expression

Table 2. Results of meta-analysis

Polymorphism	OR (95% CI)	P value	I ² (%)	P value
rs1799987	1.38 (1.17-1.63)	0.0001	78	0.00001
Asian	1.68 (1.33-2.11)	<0.0001	63	0.02
Caucasian	1.20 (1.00-1.43)	0.05	74	0.0008
T1D	1.10 (1.00-1.20)	0.05	27	0.22
T2D	1.79 (1.58-2.02)	<0.00001	41	0.13
EDN	1.41 (1.16-1.71)	0.0005	81	0.00001
ADN	1.30 (0.90-1.90)	0.17	74	0.02
rs333	1.36 (0.79-2.33)	0.27	88	0.00001
Asian	1.55 (0.54-4.44)	0.42	92	0.0005
Caucasian	1.22 (0.66-2.28)	0.53	81	0.005
T1D	1.55 (0.54-4.44)	0.42	92	0.0005
T2D	1.22 (0.66-2.28)	0.53	81	0.005
EDN	0.88 (0.52-1.47)	0.62	0	0.86
ADN	1.46 (0.76-2.78)	0.26	89	0.0001

EDN, Established diabetic nephropathy; ADN, Advanced diabetic nephropathy.

by peripheral blood mononuclear cells [14]. The rs1799987 homozygosity was associated with increased promoter activity and enhanced protein expression by CD4+ T cells [15]. Therefore, CCR5 rs1799987 polymorphism might influence the risk DN.

de Oliveira et al. suggested that there is involvement of the CCR5 rs1799987 polymorphism in susceptibility to the different forms of chronic Chagas disease [16]. Han et al. found that CCR5 gene polymorphism was a genetic risk factor for radiographic severity of rheumatoid arthritis [17]. Zhao et al. indicated that CCR5 rs1799987 polymorphism associated with increased risk of ischemic stroke in a Chinese Han population [18].

Although we attempted to avert statistical bias in our study, there were always some shortcomings in this meta-analysis. Firstly, through literature searches, we found that 9 articles could be used to this meta-analysis, so the accuracy of combined analysis might be affected. Secondly, all of the case-control studies were conducted in Asians and Caucasians; thus, our results may be applicable only to these ethnic groups. Thirdly, the inconsistency of the base line characteristics between the studies, such as age and gender, might increase the selection bias. Finally, the publication biases cannot be completely excluded due to

that all of the included studies were mainly relying on observation.

In conclusion, this meta-analysis suggests that individuals with CCR5 rs1799987 polymorphism might have an increased DN risk.

Disclosure of conflict of interest

None.

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