ORIGINAL ARTICLE

# Association of chemokine ligand 5/chemokine receptor 5 gene promoter polymorphisms with diabetic microvascular complications: A meta-analysis

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## ABSTRACT

**Aims/Introduction:** Chemokine ligand 5 (CCL5) is a member of the CC-chemokine family expressed in various organs. It contributes to the migration of monocytes/macrophages into injured vascular walls by binding with its receptor chemokine receptor 5 (CCR5). Many studies have accessed the association between CCL5/CCR5 gene promoter polymorphisms and diabetic microvascular complications (DMI). However, the results are conflicting and inconclusive. The aim of the present study was to evaluate the association more precisely.

**Materials and Methods:** Trials were retrieved through PubMed, Embase, Medline, China National Knowledge Infrastructure, Web of Science and Cochrane database without restrictions on language. The pooled odds ratio (OR) and 95% confidence interval (CI) were used to describe the strength of association with DMI.

**Results:** Data were obtained from 11 case–control studies that included 2,737 DMI patients and 2,435 diabetic control subjects. In the overall analysis, the CCL5-403 G/A and CCL5-28 C/G gene polymorphisms were not significantly associated with the risk of DMI. However, CCR5-59029 G/A was an independent risk factor of DMI in a dominant model (OR 1.77, 95% CI 1.06–2.97). Subgroup analysis showed that the risk of the CCR5 59029A-positive genotype was significant in Asians (OR 2.08, 95% CI 1.68–2.57). In addition, the CCR5 59029A-positive genotype was associated with increased risk of albuminuria.

**Conclusions:** There were no associations of CCL5 gene promoter polymorphism with the risk of DMI. However, the 59029A polymorphism in CCR5 might affect individual susceptibility for DMI.

## INTRODUCTION

The prevalence of diabetes mellitus has increased rapidly in recent years. Diabetic microvascular complications (DMI), such as diabetic nephropathy (DN) and diabetic retinopathy (DR), are the most devastating clinical manifestations of diabetes. The risk factors of DMI include hyperglycemia, hypertension and genetic factors. Although hypoglycemic and antihypertensive treatments were inversely related to DMI in some cases, many patients still developed DMI even when their blood glucose and blood pressure reached normal levels[1,2]. These patients might have genetic risk factors associated with DMI. In addition, DMI can be considered as a cytokine-associated innate immune response[2], and downregulation of specific cytokines could reduce the incidence of DMI[3,4]. Identification of cytokines with higher risks will be useful for early preventive care of DMI.

Chemokine ligand 5 (CCL5), also known as regulated on activation normal T expressed and secreted (RANTES), is a member of the C-C chemokine family, and is mainly expressed on CD8 + T cells[3]. Chemokine ligand 5 has the ability to recruit, activate and costimulate T cells, and thus mediate innate and adaptive immune responses[5]. The cognate receptor of CCL5, chemokine receptor 5 (CCR5), a G-coupled seven-transmembrane chemokine receptor, is a member of the β-chemokine receptor family, and mainly expressed on T cells and macrophages[3,5–7]. Genetic inactivation of CCR5 is associated with the reduction of monocytes/macrophages into the injured vessels, which is one of the earliest events in the pathogenesis of DMI[1]. In addition, several studies have shown that CCL5/CCR5 expression was increased in patients with DN or DR, and their aberrant expression was associated with renal inherent injury or the formation of intraocular neovascularization[8,9]. Thus, the mutations in the CCL5 or CCR5 gene might be the pathogenic determinant of DMI.

A screening of the CCL5/CCR5 gene for sequence variants identified several genetic variants. The most common polymorphisms are CCL5-403 G/A (G to A alteration), CCL5-28 C/G (C to G alteration) and CCR5 59029 G/A (G to A alteration). However, studies regarding CCL5-403 G/A, CCL5-28 C/G, CCR5 59029 G/A and DMI risk are controversial. Some researchers have shown that the CCL5 and CCR5 genes variants could be susceptibility factors for DMI[1,10–16]. Yet, the results have not been verified in other studies[3,9,17]. The primary aim of the present study was to derive a more precise evaluation of the associations between the CCL5/CCR5 genes variants and the risk of DMI. The secondary analysis was to identify factors that might affect the association strength between the CCL5/CCR5 gene polymorphism and the risk of DMI.

## MATERIALS AND METHODS
### Search strategy
We searched PubMed, Embase, Medline, China National Knowledge Infrastructure (CNKI), Web of Science, Cochrane database and reference lists of relevant studies. Using the terms 'CCR5', 'CCL5' and 'RANTES' in combination with 'diabetes mellitus' or 'DMI' or 'DN' or 'diabetes kidney disease' or 'DR' or 'diabetic foot' or 'variant' or 'mutation' or 'polymorphism'. No restriction was set on language or whether the articles had been published. We also checked the references of these articles to screen out other relevant publications. In addition, we carried out this meta-analysis in accordance with the Preferred Reporting Items for Reviews and Meta-Analyses (PRISMA) statement.

### Study selection criteria
Eligible articles met the following criteria: (i) case–control studies: patients with DN and/or DR as the case group, diabetic patients without complications as the control group; (ii) prospective cohort studies; (iii) studies related to the CCL5-

403 G/A, CCL5-28 C/G, and CCR5 59029G/A polymorphism and DMI risk. Exclusion criteria included: (i) non case–control studies; (ii) the control group including non-diabetic patients; (iii) diabetic patients combined with serious complications, such as malignancies and severe cardiac dysfunction.

### Data extraction
Two investigators (ZZ and JD) reviewed the eligibility of the studies and extracted frequency difference of genetic variants in DMI. When more than one study by the same author was included, only the most complete study was included in our research. The data extracted from articles including the author name, year of article, ethnic origin, numbers of genotype cases and control subjects, age, sex, glycated hemoglobin levels and subtype of diabetes mellitus.

### Data synthesis and statistical analysis
The Hardy–Weinberg equilibrium was tested by the $\chi^2$-test. The strength of genetic variants and DMI association were evaluated by odds ratio (OR) and 95% confidence interval (CI). If the association showed heterogeneity ($I^2 > 25\%$), the random effects models were merged. Furthermore, sensitivity analysis was carried out to determine the source of heterogeneity, thereby evaluating whether the heterogeneity markedly influenced the results. The association between CCL5/CCR5 gene mutation and DMI were analyzed by the following methods: CCL5-403 G/A (GA + AA vs GG; AA vs GA + GG), CCL5-28 C/G (GC + CC vs GG; CC vs GC + GG) and CCR5-59029G/A (GA + AA vs GG, AA vs GA + GG). The risk frequency of A allele (CCL5-403 G/A and CCR5-59029G/A) and G allele (CCL5-28 C/G) were also calculated in these case–control groups. The subtype of diabetes mellitus (type 1 diabetes or type 2 diabetes), the specific type of DMI (DN or DR), the stage of DN (microalbuminuria, macroalbuminuria or end-stage renal disease [ESRD]) and ethnicity (Caucasians or Asians) were selected for stratified analysis. The Egger's test and Rosenthal's fail-safe number ($N_{fs}$) were used to test the publication bias. Values of $P < 0.05$ were considered as significant differences. The significance of $N_{fs}$ was set at 0.05 for each meta-comparison. If the calculated $N_{fs}$ value was smaller than the number of studies, the meta-analysis results might show a risk of publication bias. The formula $N_{fs0.05} = (\Sigma Z/1.64)^2 - k$ ($k$ is the number of articles included in this research). All analyses were carried out using two statistical software programs: Review Manager 5.2 (Cochrane Collaboration, Oxford, UK) and STATA 10.0 (StataCorp, College Station, TX, USA). This meta-analysis was carried out using the Mantel–Haenszel statistical method and sample size comparing the weighted mean difference.

## RESULTS
### Study characteristics
Our research yielded 601 reports of potentially relevant studies. After screening, 11 case–control studies and two conference abstracts were eligible, including nine articles in English and

two articles in Chinese. The study selection process is shown in Figure 1. A total of 2,737 DMI patients and 2,435 diabetic controls were enrolled, ten studies focused on the CCR5-59029G/A polymorphism, three on CCL5-28 C/G polymorphism and four focused on CCL5-403 G/A polymorphism. The main characteristics of the included studies are listed in Table S1. Among these studies, ten evaluated albuminuria, three evaluated microalbuminuria, five evaluated macroalbuminuria and two evaluated ESRD (Table S2).

### Main meta-analysis results
*Risk factor analysis of chemokine ligand 5-403G/A, chemokine ligand 5-28 C/G and diabetic microvascular complications*
The fixed effects model was used because the heterogeneity of data was less than 30%. It showed that CCL5-403 G/A, CCL5-28 C/G genetic polymorphisms had no significant correlation

with DMI (OR 1.00, 95% CI 0.82–1.21 for CCL5-403 G/A; OR 1.02 95% CI 0.79–1.33 for CCL5-28 C/G). Subgroup analysis was carried out for the subtype of diabetes mellitus and ethnicity. However, exclusion of the two variables individually did not generate significant changes for the pooled association (Table 1).

*Risk factor analysis of chemokine receptor 5-59029G/A and diabetic microvascular complications*
When CCR5-59029 GA + AA genotypes were set as dominant risk factors, the result showed that CCR5 59029 GA + AA genotypes were associated with increased DMI risk (OR 1.77, 95% CI 1.06–2.97, $P = 0.03$). When the AA genotype was set as the recessive risk factor, no significant correlation was found between the AA genotype and DMI risk (OR 1.28, 95% CI 0.75–2.17, $P = 0.36$). These results suggested that the CCR5
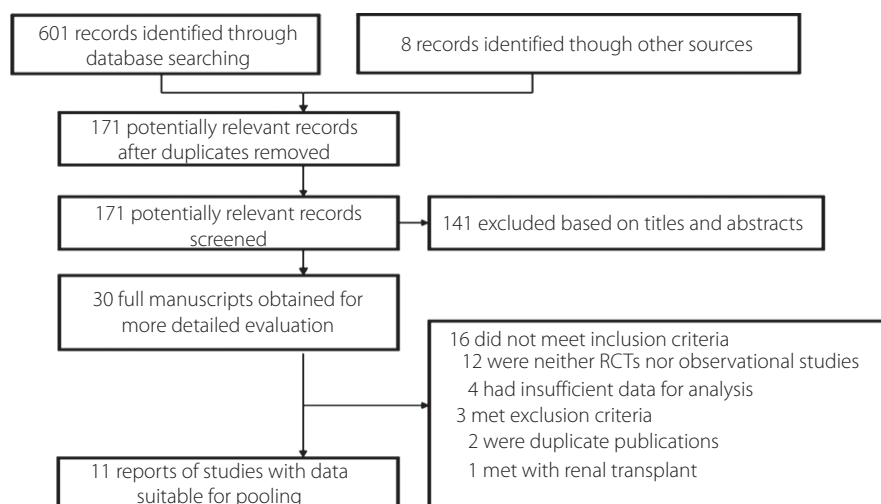


**Figure 1** | Flow chart of the systematic search process. RCTs, randomized controlled trials.

**Table 1** | Meta-analysis of chemokine ligand 5 (CCL5) polymorphism and the risk of diabetic microvascular complications with a dominant model

| Category | $n$ | Participants, $n$ (cases/controls) | Heterogeneity | | OR (95% CI) | Z-test |
|---|---|---|---|---|---|---|
| | | | $P_h$ | $I^2$ (%) | | |
| Four studies for the CCL5-403 G/A polymorphism | | | | | | |
| Overall | 4 | 797/1064 | 0.26 | 26 | 1.00 (0.82–1.21)† | $Z = 0.02$; $P_Z = 0.98$ |
| Adjustment by subtypes of diabetes | | | | | | |
| Type 1 diabetes mellitus | 1 | 267/440 | NA | NA | 1.21 (0.87–1.68) | $Z = 1.14$; $P_Z = 0.26$ |
| Type 2 diabetes mellitus | 3 | 530/624 | 0.36 | 1 | 0.90 (0.71–1.15)† | $Z = 0.85$; $P_Z = 0.39$ |
| Adjustment by ethnicity | | | | | | |
| Caucasian | 1 | 267/440 | NA | NA | 1.21 (0.87–1.68) | $Z = 1.14$; $P_Z = 0.26$ |
| Asian | 3 | 530/624 | 0.36 | 1 | 0.90 (0.71–1.15)† | $Z = 0.85$; $P_Z = 0.39$ |
| Three studies for the CCL5-28C/G polymorphism | | | | | | |
| Overall (all T2DM) | 3 | 508/659 | 0.88 | 0 | 1.02 (0.79–1.33)† | $Z = 0.17$; $P_Z = 0.87$ |

†Fixed-effects model. $P_Z < 0.05$, shows a significant association. CI, confidence interval; NA, not available; OR, odds ratio; $P_h$, P-values for heterogeneity of Q-test; T2DM, type 2 diabetes mellitus.
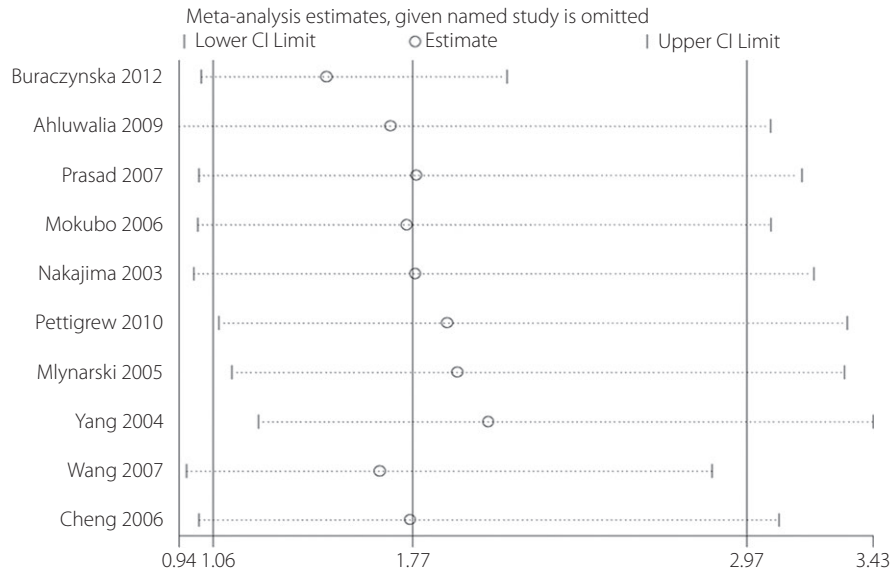
**Figure 2** | The sensitivity analysis for the association of the CCR5-59029 G/A polymorphism with the risk of diabetic microvascular complications. CI, confidence interval.

59029A-positive genotype (G/A or A/A) was an independent risk factor of DMI.

We found significant heterogeneity of the CCR5 59029A-positive genotype study ($I^2 = 91\%$). Quantitative analysis showed that heterogeneity arose from the trials of Ahluwalia et al.[13], Buraczynska et al.[1], Pettigrew et al.[3], Mlynarski et al.[11], Yang et al.[9] and Cheng et al.[16] (Figure 2). The heterogeneity disappeared ($I^2 = 0\%$) after excluding these studies. The trials published by Pettigrew et al.[3], Mlynarski et al.[11] and Yang et al.[9] evaluated the risk of microvascular complications in type 1 diabetes mellitus, while the trails published by Ahluwalia et al.[13], Buraczynska et al.[1] and Cheng et al.[16] analyzed the risk of microvascular complications in type 2 diabetes mellitus. Thus, the type of diabetes mellitus was selected for stratification analysis. The result showed that the CCR5 59029A-positive genotype was significantly associated with microvascular complications in type 2 diabetes mellitus (OR 2.61, 95% CI 1.57–4.36, $P < 0.05$), but not in type 1 diabetes mellitus (OR 0.82, 95% CI 0.56–1.20, $P > 0.05$; Figure 3). Both the Cochran $Q$-test and estimate of $I^2$ showed significant heterogeneity among these studies. The frequencies of the CCR5 variants from each study are listed in Table S3.

To further evaluate the cause of heterogeneity in microvascular complications of type 2 diabetes mellitus ($I^2 = 85\%$), the ethnicity, the specific type of DMI and the stage of DN were selected for subgroup analysis. The results showed that ethnicity and the stage of DN were correlated with heterogeneity. After excluding the Caucasian population, the heterogeneity disappeared ($I^2 = 0\%$), and a significant association was found in Asians (OR 2.07, 95% CI 1.68–2.56, $P < 0.00001$). The CCR5 59029A-positive genotype significantly increased the risk of

albuminuria (OR 1.68, 95% CI 1.15-2.44, $P < 0.05$ for microalbuminuria; OR 2.70, 95% CI 1.07–6.83, $P < 0.05$ for macroalbuminuria). As only one trial was on ESRD, we were unable to explore the relationship between CCR5 59029A-positive genotype and risk of ESRD. The details are listed in Table 2.

## Publication bias

Publication biases were tested for all the outcomes of the included studies. From the funnel plot, no significant publication bias was found among these studies (Figures S1–S3). We also calculated the $N_{fs}$ 0.05 for CCL5-403 G/A, CCL5-28 C/G and CCR5-59029G/A. The $N_{fs}$ 0.05 value of each comparison was greater than the number of studies included in our research.

## DISCUSSION

Genetic epidemiological studies of single nucleotide polymorphisms can explore the association between the candidate gene and disease risks. In the present meta-analysis, we found no positive association of the CCL5 gene polymorphism and the risk of DMI. However, the 59029A polymorphism in CCR5 might affect individual susceptibility for DMI, and the CCR5 59029A-positive genotype was associated with increased DMI risk in a subgroup ('type 2 diabetes mellitus' or 'Asians', respectively). In addition, the CCR5 59029 A-positive genotype was associated with increased risk of microalbuminuria and macroalbuminuria.

Ethnic differences might partly attribute to the interaction between the genetic and geographical environment[7,18]. Considering that DMI is a complex etiology involving the combined effects of environmental and genetic factors, different ethnic
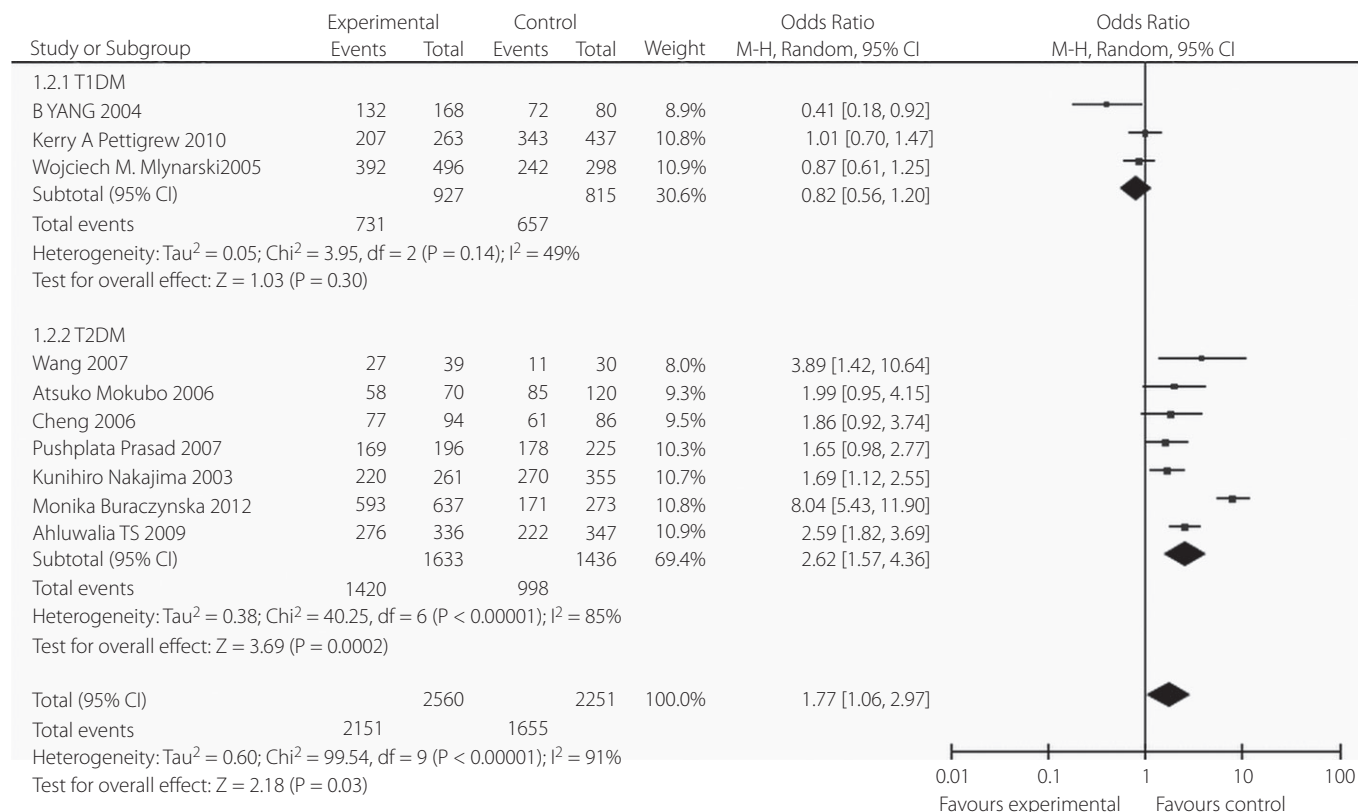
**Figure 3** | Forest plots of the meta-analysis for CCR5-59029G/A polymorphism associated with the subtypes of diabetes mellitus. CI, confidence interval; df, degrees of freedom; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

**Table 2** | Meta-analysis of chemokine ligand 5 (CCL5) promoter polymorphism and the risk of diabetic microvascular complications with a dominant model

| Category | $n$ | Participants, $n$ (cases/controls) | Heterogeneity | | OR (95% CI) | $Z$-test |
|---|---|---|---|---|---|---|
| | | | $P_h$ | $I^2$ (%) | | |
| Overall | 10 | 2560/2251 | 0.00 | 91 | 1.77 (1.06–2.97) | $Z = 2.18$; $P_Z = 0.02$ |
| Adjustment by subtypes of DMI | | | | | | |
|   Diabetic nephropathy | 8 | 2323/2251 | 0.00 | 91 | 1.90 (1.10–3.27) | $Z = 2.31$; $P_Z = 0.02$ |
|   Diabetic retinopathy | 2 | 237/353 | 0.00 | 91 | 1.10 (0.16–7.35) | $Z = 0.10$; $P_Z = 0.91$ |
| Adjustment by stage of DN | | | | | | |
|   Microalbuminuria | 3 | 281/471 | 0.95 | 0 | 1.68 (1.15–2.44)† | $Z = 2.71$; $P_Z = 0.007$ |
|   Macroalbuminuria | 5 | 1420/1085 | 0.00 | 93 | 2.70 (1.07–6.83) | $Z = 2.70$; $P_Z = 0.04$ |
|   ESRD | 1 | 196/225 | NA | NA | 1.65 (0.98–2.77) | $Z = 1.90$; $P_Z = 0.06$ |
| Adjustment by subtypes of diabetes mellitus | | | | | | |
|   Type 1 diabetes mellitus | 3 | 927/815 | 0.14 | 49 | 0.82 (0.56–1.20) | $Z = 1.02$; $P_Z = 0.31$ |
|   Type 2 diabetes mellitus | 7 | 1633/1436 | 0.00 | 85 | 2.61 (1.57–4.36) | $Z = 5.05$; $P_Z < 0.00$ |
| Adjustment by ethnicity | | | | | | |
|   Caucasian | 4 | 1564/1088 | 0.00 | 96 | 1.33 (0.39–4.47) | $Z = 0.47$; $P_Z = 0.63$ |
|   Asian | 6 | 996/1163 | 0.44 | 0 | 2.08 (1.68–2.57)† | $Z = 6.78$; $P_Z = 0.00$ |

†Fixed-effects model; $P_Z < 0.05$, shows a significant association. DMI, diabetic microvascular complications; DN, Diabetic nephropathy; ESRD, end-stage renal disease; NA, not available; $P_h$, P-values for heterogeneity of Q-test.

populations have different frequencies of alleles and genetic backgrounds, which could affect the risk of DMI[18]. In our research, the frequency of the 'A' allele in the Caucasian population (case group: mean 0.0035, range 0.002–0.006; control group: mean 0.0057, range 0.002–0.015) was lower than that in the Asian population (case group: mean 0.0123, range

0.004–0.029; control group: mean 0.008, range 0.002–0.0105), which could be one of the sources for variety in genetic susceptibility between different ethnic groups. In addition, ethnicity might correlate with the curative activities, such as drug use or target of blood glucose[18]. Mokubo et al.[10] found that the CCR559029 A (+) genotype and 10-year mean glycated hemoglobin value were positively correlated with DMI risk among the Asian patients. However, Mlynarski et al.[11] failed to find such a correlation with Caucasians. Thus, curative activities might account for the inconsistency of the studies.

There was no significant association of the CCL5 gene polymorphism and the risk of DMI. Some studies pointed out that their relationship might correlate with the stage of DN or sex (male)[11,12]. As only a few studies are available, we were not able to confirm these conclusions. In addition, the present study only focused on DMI, and did not evaluate the association of the CCL5 gene polymorphism and other disease, such as atherosclerosis, multiple sclerosis and atopic asthma[19–21]. The potential role of the CCL5 gene polymorphism might be masked by other gene–gene or gene–environment interactions.

Diabetic microvascular complications is caused by a combination of the effects of multiple genetic and environmental risk factors. Comprehensive research of the multiple loci will explore a novel biological insight for us[22–25]. In the present study, we found that, in Asians, the CCR5 59029A-positive genotype is a significant susceptibility factor for DMI, which implies that genetic variant rs1799987 in CCR5 could increase the risk of DMI in Asians. Therefore, in the Asian population, CCR5 might present a new target for the early preventive detection of DMI.

Although Nazir et al.[26] reported that CCR5 genetic variants showed a significant association with DN, the role of CCR5 in DMI remains unclear. Compared with the study by Nazir et al.[26], the present study used a higher number of articles for this meta-analysis, and carried out subtype and sensitivity analysis to evaluate the causes of heterogeneity. The association between the CCR5 gene polymorphism and the stage of DN were also analyzed in the present study. In addition, the publication biases were tested for all the outcomes of the included studies in the present study. The previous study[26] was unable to calculate the publication bias because of a lack of studies for CCL5 C-28G genetic variants (i.e., less than three studies).

Several limitations to the present study should be considered. First, the sample size of the CCL5 polymorphism is relatively small, and additional studies are required to confirm the conclusion. Second, we focused only on the single nucleotide polymorphisms on CCL5/CCR5 promoter, while the other single nucleotide polymorphisms in the CCL5/CCR5 gene should be studied to clarify the role of CCL5/CCR5 in DMI. Third, in this meta-analysis, most of the Caucasian studies involved type 1 but not type 2 diabetes. A lack of association between the CCR5 polymorphism and the risk of DMI in Caucasians might partially result from the type of diabetes, rather than ethnicity.

## DISCLOSURE

The authors declare no conflict of interest.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1 |** Funnel plot of publication bias for the association of the CCL5-403 G/A polymorphism with the risk of diabetic microvascular complications.

**Figure S2 |** Funnel plot of publication bias for the association of the CCL5-28C/G polymorphism with the risk of diabetic microvascular complications.

**Figure S3 |** Funnel plot of publication bias for the association of the CCR5-59029G/A polymorphism with the risk of diabetic microvascular complications.

**Table S1 |** Main characteristics of the studies included in the meta-analysis.

**Table S2 |** The description of diabetic nephropathy staging studies included in the subgroup-analyses.

**Table S3 |** The frequencies of the CCR5-59029 A positive genotype from each study in the subgroup analyses.