

# Meta – analysis of association between K469E polymorphism of the ICAM–1 gene and retinopathy in type 2 diabetes

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## Abstract

• **AIM:** To collectively evaluate the association of intercellular adhesion molecule–1 (ICAM–1) gene K469E polymorphism (rs5498) with diabetic retinopathy (DR) in patients with type 2 diabetic mellitus (T2DM).

• **METHODS:** Overall review of available literatures relating K469E polymorphism to the risk of DR was conducted on 4 electronic databases. Meta–analysis was performed by Stata 12.0 to calculate pooled odds ratios (ORs). Potential sources of heterogeneity and bias were explored.

• **RESULTS:** Seven studies with genotype frequency data including 1120 cases with DR and 956 diabetic controls free of DR were included. Meta –analysis did not show significant association of K469E polymorphism with DR ( $P>0.05$ ). A statistically significant association was detected between the K469E polymorphism and proliferative DR (PDR) in Asians only in dominant model (GG+AG vs AA) with pooled OR of 0.729 (95%CI: 0.564–0.942,  $P=0.016$ ,  $I^2_{\text{heterogeneity}}=0.143$ ), however, this association was not detected in recessive model (AG+AA vs GG; OR=1.178, 95%CI: 0.898–1.545,  $P=0.236$ ,  $I^2_{\text{heterogeneity}}=0.248$ ) or allelic model (G vs A; OR=0.769, 95% CI: 0.576–1.026,  $P=0.074$ ,  $I^2_{\text{heterogeneity}}=0.094$ ). No publication bias was found by Funnel plot, Begg’s and Egger’s test.

• **CONCLUSION:** This research found no statistically significant association between ICAM –1 gene K469E polymorphism and DR in patients with T2DM, but showed significant association of the K469E polymorphism with PDR in Asian diabetic patients only in dominant model. Further investigation would be required to consolidate the conclusion.

• **KEYWORDS:** K469E polymorphism; rs5498; intercellular

adhesion molecule-1; diabetic retinopathy; type 2 diabetes; Meta-analysis

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## INTRODUCTION

Diabetic retinopathy (DR), one of the most threatening microvascular complications in diabetic patients, leads to vision damages in approximately 75% of individuals with at least 15y of diabetic duration<sup>[1]</sup>. DR is characterized by loss of pericytes, endothelial cell dysfunction, blood-retinal barrier breakdown, capillary non-perfusion, microaneurysm, haemorrhage and neovascularization. Inflammation is believed to play an important role in the pathogenesis of DR<sup>[2]</sup>.

Genetic factors were found to be responsible for nearly 20% development of DR besides duration of diabetics and bad control of blood glucose<sup>[3]</sup>. A number of genes had been observed to be the candidate genetic factors predisposing the development of DR<sup>[4,5]</sup>. Published observation suggested a crucial role of intercellular adhesion molecule-1 (ICAM-1) in the development of DR by mediating inflammation process and endothelial cell function<sup>[6]</sup>. The K469E polymorphism (rs5498) of the ICAM-1 gene results in a non-conservative change from lysine to glutamic acid in the fifth immunoglobulin-like domain 5 which is important for the activity of the ICAM-1 protein<sup>[7]</sup>.

A sum of studies reported the association between K469E polymorphism of ICAM-1 gene and DR in type 2 diabetics with controversial results<sup>[8-14]</sup>. There are studies suggesting the high frequency of AA genotype in DR<sup>[8,11]</sup>, whereas the others presented an opposite result with GG to be a risk factor for DR<sup>[9]</sup>. To understand the role of K469E polymorphism of ICAM-1 gene in the pathogenesis of DR, we conducted a Meta-analysis to summarize the results by calculating pooled odds ratios (ORs).

## SUBJECTS AND METHODS

**Search Strategy** We performed an overall literature review on four electronic databases: PubMed (National Center for Biotechnology Information), ISI (Web of Knowledge), Embase, CNKI (China National Knowledge Internet), and

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**Table 1** Baseline characteristics of patients enrolled in selected studies

No.	Study	Year	Country	Patients sources	No. of case/control	Age (a)	BMI (kg/m <sup>2</sup> )	Duration of T2DM (a)	Sex (male) cases/controls	HWE (P)
1	Vinita <i>et al</i> <sup>[8]</sup>	2012	India	Hospital	199/157	58.81±8.63/64.32±9.01	23.79±5.36/25.33±7.78	17.74±5.45/18.44±6.18	128/98	>0.05
2	Balalabubu <i>et al</i> <sup>[10]</sup>	2010	India	Hospital	345/359	57±9/59±11	-	14±9/14±9	242/208	>0.05
3	Zhou <i>et al</i> <sup>[13]</sup>	2010	China	Hospital	102/120	55.6±8.8/56.3±9.4	-	-	52/62	>0.05
4	Petrovic <i>et al</i> <sup>[9]</sup>	2008	Slovenia	Hospital	195/143	65.2±9.9/66.9±11.5	28.1±4.4/27.7±4.4	19.2±8.6/16.5±6.6	91/57	>0.05
5	Chen and Yu <sup>[14]</sup>	2007	China	Hospital	66/87	62.42±10.37/61.63±8.24	24.93±3.65/25.03±3.16	-	31/45	>0.05
6	Liu <i>et al</i> <sup>[11]</sup>	2006	China	Hospital	132/40	PDR: 55.0±12.41 NPDR: 63.7±7.90 DWR: 61.5±11.53	-	PDR: 14.7±3.48 NPDR: 12.9±2.61 NDR: 13.7±1.90	44/19	>0.05
7	Kamiuchi <i>et al</i> <sup>[12]</sup>	2002	Japan	Hospital	81/50	64.3±8.9/64.1±9.1	-	17/16	35/24	>0.05

BMI: Body mass index; T2DM: Type 2 diabetic mellitus; HWE: Hardy-Weinberg equilibrium; PDR: Proliferative diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy; DWR: Diabetic patient without retinopathy; -: No data available.

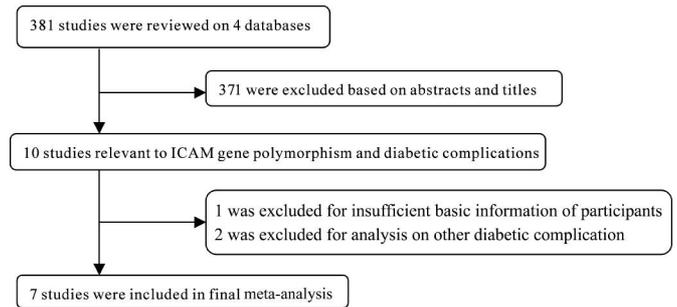
included related studies published in English and Chinese before November 2013. The key words were "diabetic retinopathy", "diabetic complication", "K469E", "rs5498", "ICAM-1", "gene", "genetic", and "intercellular adhesion molecule-1". Manual search was used to identify targets in references of related articles. Editorials, case reports and review articles were excluded. The process of literatures selection was shown in Figure 1.

**Inclusion Criteria** 1) Case-control or cohort studies published about the relationship of K469E polymorphism of ICAM-1 gene and DR in patients with type 2 diabetic mellitus (T2DM). 2) Determination of DR was made by ophthalmoscopy or fundus photography after pupil dilation. 3) Adequate information about the genotype and allele were available. 4) Languish in English or Chinese.

**Exclusion Criteria** 1) Insufficient data in frequencies of genotype and allele. 2) Insufficient information about baseline characteristics of participants. 3) Language other than English and Chinese. 4) Genotype distribution deviates from the Hardy-Weinberg equilibrium (HWE) in the control group.

**Data Extraction** For enrolled articles, the following information was extracted, including the author's last name, inclusion criteria of cases and controls, year of publication, ethnics, baseline characteristics of cases, genotype and allele frequencies of cases and controls.

**Statistical Analysis** The distributions of the genotypes in the control groups of selected studies were examined using Pearson's Chi-square test, and *P* value >0.05 suggested accordance with the HWE. Software Stata 12.0 was used to perform Meta-analysis by calculating pooled ORs and 95% confidence interval (CI) in dominant (GG+AG *vs* AA), recessive (AG+AA *vs* GG) and allelic models (G *vs* A), respectively. Inter-group heterogeneity was evaluated by inconsistency index (*I*<sup>2</sup>) and *P*<sub>heterogeneity</sub>. While *P*<sub>heterogeneity</sub> <0.1, pooled OR was estimated by using random-effect model otherwise using fix-effect model. Sensitivity analyses were performed by omitting each study to identify possible study contributing to the heterogeneity. Two-sided *P* value <0.05 means statistically significant. Funnel plot, Begg's and Egger's tests were applied to explore potential publication



**Figure 1** Chart flow of publications selection process.

**Table 2** Genotype and allele frequencies of cases and controls

No.	Study	Frequencies of genotype					
		GG		AG		AA	
		DR	DWR	DR	DWR	DR	DWR
1	Vinita <i>et al</i> <sup>[8]</sup>	47	44	92	84	60	29
2	Balalabubu <i>et al</i> <sup>[10]</sup>	80	86	162	174	103	99
3	Zhou <i>et al</i> <sup>[13]</sup>	21	21	44	45	37	54
4	Petrovic <i>et al</i> <sup>[9]</sup>	52	22	96	77	47	44
5	Chen and Yu <sup>[14]</sup>	1	6	20	27	45	54
6	Liu <i>et al</i> <sup>[11]</sup>	11	9	40	15	81	16
7	Kamiuchi <i>et al</i> <sup>[12]</sup>	12	10	35	30	34	10

DR: Diabetic retinopathy (including PDR and NPDR); DWR: Diabetic patient without retinopathy.

bias. A significance level of 0.05 was used as an indication for the presence of potential publication bias.

## RESULTS

Figure 1 shows the process of literatures selection. In total, seven studies with genotype frequency data from 1120 cases of DR and 956 diabetic patients without retinopathy (DWR) were included, involving 6 Asian studies and 1 Caucasian study. Table 1 shows the baseline characteristics of patients enrolled in selected studies. The frequencies of genotypes in control groups were all in the HWE. The inclusion criteria of cases varied in different studies. Two of them included proliferative DR (PDR)<sup>[9-10]</sup>. One included severe sight threatening DR including severe non-PDR (NPDR), PDR or clinically significant macular edema<sup>[8]</sup>, which was attributed into PDR subgroup in the following Meta-analysis. The remaining 4 studies involved both PDR and NPDR<sup>[11-14]</sup>, and one of them had separate data for PDR and NPDR<sup>[11]</sup>. The genotype and allele information of cases and controls were shown in Table 2.

**Table 3 ORs and heterogeneity results in different models for DR vs DWR**

Model	Groups	Studies included	Numbers of DR/DWR	Calculating method	Pooled OR (95%CI)	P	I <sup>2</sup> (%)	P <sub>heterogeneity</sub>
Dominant model GG+AG vs AA	Overall	7	1120/956	Random	0.767 (0.528-1.113)	0.162	69.4	0.003
	Asian	6	925/813	Random	0.687 (0.466-1.014)	0.059	65.1	0.014
	Asian sensitivity analysis	5	823/693	Random	0.603 (0.419-0.868)	0.006	50.9	0.086
Recessive model AG+AA vs GG	Overall	7	1120/956	Random	1.120 (0.747-1.679)	0.583	61.1	0.017
	Asian	6	925/813	Fixed	1.187 (0.934-1.508)	0.161	34.3	0.179
	Asian sensitivity analysis	5	823/693	Fixed	1.253 (0.970-1.619)	0.085	36.2	0.180
Allelic model G vs A	Overall	7	1120/956	Random	0.840 (0.633-1.115)	0.227	76.3	0.000
	Asian	6	925/813	Random	0.765 (0.583-1.004)	0.053	67.2	0.009
	Asian sensitivity analysis	5	823/693	Random	0.696 (0.534-0.907)	0.007	58.0	0.049

Overall: All included studies; Asian: Studies in Asian; Asian sensitivity analysis: Sensitivity analysis of studies in Asians; DR: Diabetic retinopathy (including PDR and NPDR); DWR: Diabetic patient without retinopathy; Fixed: Fixed effect model; Random: Random effect model; OR: Odds ratio; P: P value of Meta-analysis; I<sup>2</sup>: Inconsistency index; P<sub>heterogeneity</sub>: P value of heterogeneity.

In the Meta-analysis comparing DR with DWR, no association was found between the K469E polymorphism and DR ( $P > 0.05$ ; Table 3, Figure 2). Since there were 6 studies involving Asian, we performed subgroup analysis in Asians and found marginal associations of K469E polymorphism with DR in dominant model ( $P = 0.059$ ) and allelic model ( $P = 0.053$ ), and those associations were strengthened and became statistically significant in sensitivity analysis with Zhou *et al*'s [13] study removed (dominant model:  $P = 0.006$ ; allelic model:  $P = 0.007$ ; Table 3).

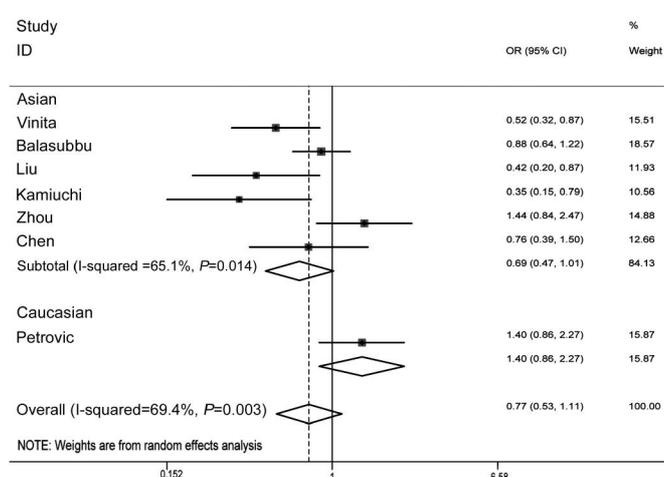
For Meta-analysis comparing PDR with DWR, three studies that did not separate PDR and NPDR [12-14] was excluded from the analysis. Available data from other studies suggested a statistical significant association between the K469E polymorphism and PDR in Asian subgroup with pooled OR of 0.729 (95%CI: 0.564-0.942,  $P = 0.016$ ) in the dominant model (Figure 3), however, no association was detected in recessive model (OR=1.178, 95%CI: 0.898-1.545,  $P = 0.236$ ) and allelic model (OR=0.769, 95%CI: 0.576-1.026,  $P = 0.074$ ) (Table 4).

Funnel plots showed a symmetry distribution. No publication bias were detected both in Begg's and Egger's test ( $P > 0.05$ , Figure 4).

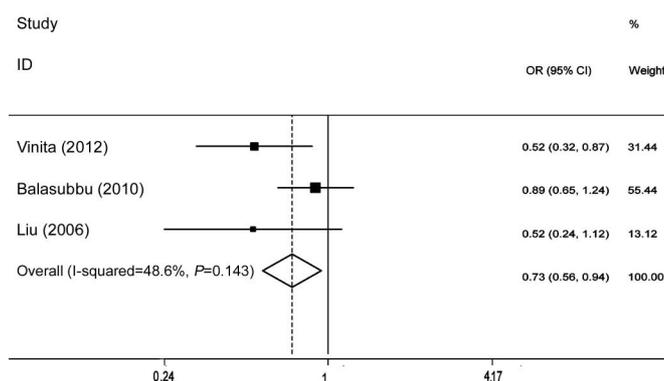
## DISCUSSION

We didn't find a significant overall association between K469E polymorphism of ICAM-1 gene with DR in patients with T2DM. This is consistent with Su *et al*'s [15] Meta-analysis [15]. However, in their report only the studies published in English were enrolled, and this might lead to missing of studies published in other languages. In addition, Su *et al*'s [15] didn't performed subgroup analysis with regards of the severity of DR (NPDR and PDR).

Although we showed no overall associations of K469E polymorphism with DR, we detected marginal associations of K469E polymorphism with DR in Asians, and those



**Figure 2 Forest plot for Meta-analysis comparing DR with DWR in dominant model (GG+AG vs AA)** Squares indicate the study-specific OR. Horizontal lines indicate 95% CIs. Diamonds indicate summary ORs with 95% CIs.



**Figure 3 Forest plot for Meta-analysis in Asians comparing PDR with DWR in dominant model (GG+AG vs AA)** Squares indicate the study-specific OR. Horizontal lines indicate 95% CIs. Diamonds indicate summary ORs with 95% CIs.

associations were strengthened and became statistically significant in sensitivity analysis with Zhou *et al*'s [13] study removed [13]. We reevaluated the design, methodology, statistic evaluation of Zhou's *et al*'s [13] study, and found no errors.

In PDR subgroup analysis, we also detected a statistically

Table 4 ORs and heterogeneity results in different models for PDR vs DWR

Model	Groups	Studies included	Numbers of PDR/DWR	Calculating method	Pooled OR (95%CI)	P	I <sup>2</sup> (%)	P <sub>heterogeneity</sub>
Dominant model GG+AG vs AA	Overall	4	819/699	Random	0.799 (0.518-1.233)	0.310	67.6	0.026
	Asian subgroup	3	624/556	Fixed	0.729 (0.564-0.942)	0.016	48.6	0.143
Recessive model AG+AA vs GG	Overall	4	819/699	Random	1.038 (0.630-1.710)	0.885	70.7	0.017
	Asian subgroup	3	624/556	Fixed	1.178 (0.898-1.545)	0.236	28.3	0.248
Allelic model G vs A	Overall	4	819/699	Random	0.885 (0.627-1.249)	0.486	79.1	0.002
	Asian subgroup	3	624/556	Random	0.769 (0.576-1.026)	0.074	57.7	0.094

Overall: All studies in PDR subgroup; Asian: Studies in PDR relevant to Asians; PDR: Proliferative diabetic retinopathy; DWR: Diabetic patient without retinopathy; Fixed: Fixed effect model; Random: Random effect model; OR: Odds ratio; P: P value of Meta-analysis; I<sup>2</sup>: Inconsistency index; P<sub>heterogeneity</sub>: P value of heterogeneity

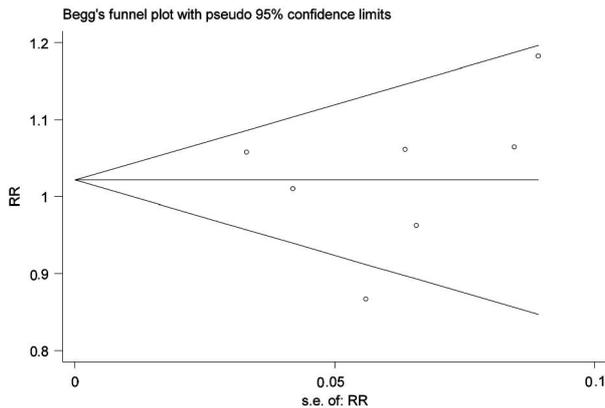


Figure 4 Funnel plot of publication bias for Meta-analysis in allelic model (G vs A). The funnel plot was symmetric and no significant publication bias was found in this Meta-analysis.

significant association of K469E polymorphism with PDR in Asian. Some factors might explain this inconsistency between overall and subgroup analysis results in our study. First, those relationships were all found in Asians, which indicated ethnicity is a possible factor for susceptibility to DR and the AA genotype seemed to be a risk factor for DR in Asian population. This is consistent with previous case-control study findings, which suggested a higher frequency of AA in Asian diabetic patients with DR but a higher frequency of GG in Caucasian<sup>[8-12]</sup>. In addition, PDR is the advanced stage of DR with typical manifestation of neovascularization and may have idiopathic pathogenesis compared with NPDR. Furthermore, in PDR subgroup, we didn't include the studies without separate published data for PDR and one study was included because its case group enrolled severe sight threatening DR that was more close to PDR, and this might influence the sample size and statistical power of Meta-analysis.

Heterogeneity is an important factor influencing the results of Meta-analysis. Although there is no absolute rule for when heterogeneity becomes important, Higgins tentatively suggested adjectives of low for I<sup>2</sup> values between 25%-50%, moderate for 50% -75% , and high for >75%<sup>[16]</sup>. When compared with fixed model, random model incorporates an estimate of heterogeneity in the weighting and typically

produces more conservative estimates of the significance of the effect, we therefore used random-effect model other than fix-effect model while I<sup>2</sup> >50% and P<sub>heterogeneity</sub> <0.1 to obtain relatively more reasonable results.

We performed funnel plot, Begg's and Egger's test and didn't find significant publication bias, which suggested the quality of this Meta-analysis is stable. However, it is still difficult to identify publication bias. We included Chen and Yu<sup>[14]</sup> study that was published in China Master's Theses Full-text Database (a sub-database of CNKI) to avoid false negative result.

We also noticed some limitations in the current study: first, we didn't enroll studies in other languages except for English and Chinese. Second, it's difficult to get unpublished separate information of NPDR and PDR in some papers, which might have some influences in subgroup analysis. Thirdly, some factors such as diabetic duration and BMI were not analyzed due to data limitations. Finally, we didn't enroll the results of unpublished studies.

In conclusion, this Meta-analysis showed no statistically significant association between ICAM-1 gene K469E polymorphism and DR in patients with T2DM, but showed significant association of the K469E polymorphism with PDR in Asian diabetic patients only in dominant model. Since K469E polymorphism locates at the coding area of ICAM-1 gene that causes missense residue change, even a moderate association of this spot with DR should cause more attentions. Further confirmation is required to verify these findings.

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Conflicts of Interest: Fan WY, None; Liu NP, None.

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