

## Original Article

# Association between *interleukin-10* rs1800872 G>T polymorphism and coronary artery disease: a case-control study and a meta-analysis

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**Abstract:** The goal of our study was to evaluate the genetic effects of interleukins genes polymorphisms on the risk of CAD through a case-control study and a meta-analysis. 826 CAD cases and 1153 CAD-free controls in east of China were collected to analyze the association of 28 SNPs in *IL1A*, *IL1B*, *IL1f7*, *IL3*, *IL7Ra*, *IL17A*, *IL18*, *IL18R*, *IL18RAP*, *IL28B*, *IL15RA*, *IL15*, *IL9*, *IL10*, *IL12A*, *IL12B*, and *IL13* genes on the risk of CAD. The distributions of all genotypes were not significantly different between CAD group and control group ( $P>0.05$ ), except for *IL1f7* rs3811047 G>A and *IL10* rs1800872 G>T genetic variants. Our results showed that *IL1f7* rs3811047 G>A, *IL28B* rs8099917 T>G and *IL10* rs1800872 G>T polymorphisms were associated with the decreased risk of CAD in various comparison models. No significant association between the other 25 SNPs and the risk of CAD were found in this population. But the meta-analysis on *IL10* rs1800872 G>T polymorphism and CAD risk, totaling 3038 CAD patients and 2732 controls, provided no convincing evidence for the genetic association (T vs. G: OR = 1.07, 95% CI 0.88-1.30,  $P = 0.510$ ), even upon stratified analysis by ethnicity (Asian and Caucasian) or control selection criteria (normal angiography and symptom investigation). The discrepant overall result from previously published studies reflects publication bias or methodological problems. Future well-designed studies with large sample size should be conducted to validate our findings.

**Keywords:** Coronary artery disease, SNP, *IL10*, meta-analysis, molecular epidemiology

## Introduction

Coronary artery disease (CAD) continues to be a leading cause of morbidity and mortality among adults globally and represents a public health challenge in both industrialized and developing countries [1]. The World Health Organization (WHO) estimated that more than 0.7 million deaths in China attributed to CAD annually [2]. CAD is a common complex disease which results from the interplay of genetic and environmental factors. Previous epidemiological studies and clinical trials provided evidence that modification of traditional risk factors for CAD, including diabetes mellitus, smoking and arterial hypertension, would lead to 30% to 40% reduction in clinical events such as myocardial infarction, ischemic heart failure and

death [3]. Moreover, apart from common risk factor, population-based studies have repeatedly reported that genetic susceptibility account for around 50% of the risk for CAD, suggesting that the host genetic variants play an important role in the occurrence and development of CAD as well [3, 4]. Studies of the genetic architecture of CAD such as GWAS have revealed a series of new candidate biomarkers that may contribute to the CAD pathogenesis, however, the functional mechanisms is remaining under investigation [5].

The inflammation is responsible for the formation, destabilization, and rupture of atherosclerotic plaques at both focal and systemic levels. Interleukins (ILs) are a group of cytokines that control cell growth and differentiation, cell

migration, inflammatory and anti-inflammatory responses of the immune system [6]. IL-37 (IL-1f7) is a newly described member of the IL-1 cytokine family, and function as a critical inhibitor of inflammation and innate immunity in various disorders [7, 8]. Accumulated evidence has showed that the IL-37 was expressed in the foam-like cells of atherosclerotic coronary and carotid artery plaques, and it may play a protective role in atherosclerosis-related diseases via inhibition of the synthesis of pro-inflammatory cytokines and suppression of macrophage activation [9, 10]. *IL28B* gene encodes cytokine IL-28B (IFN $\lambda$ 3) that belongs to the IFN $\gamma$  family. IL-28B is mainly produced by macrophages and dendritic cells (DCs) and plays a crucial role in limiting viral infections [11, 12]. Recent study have shown that IL28B was associated with hepatic lobular inflammation and fibrosis, and thus it could also be linked to the severity of systemic in particular cardiovascular alterations in CAD patients [13]. Interleukin-10 (IL-10) is a major anti-inflammatory cytokine released by Th2 cells as well as by macrophages. IL-10 is expressed in advanced human atherosclerotic plaques and played a key role in atherosclerotic plaque formation and progression [14, 15]. Besides, other inflammation cytokines were showed to be involved in the development of atherosclerosis. IL-1, IL-3, IL-12, IL-15, IL18 and IL-17A play a key role in the inflammatory response that contributes to atherosclerosis [16-21]. IL-13 regulates leukocyte recruitment and induces M2-like monocyte/macrophage differentiation in the myocardium which is associated with a favorable clinical outcome after MI [22]. IL-9 is a pleiotropic cytokine and exerted pro-atherosclerotic effects partially via induction of vascular endothelial adhesion molecule-1 (VCAM-1) expression and inflammatory cell infiltration [23]. IL-7 is a member of a sub-family of type I cytokine and plays a critical role in the promotion of clinical instability in CAD patients [24].

Basing on the biological and pathological significance of these inflammation-related genes, we conducted a case-control study to explore the associations of the potential functional polymorphisms in *IL1A*, *IL1B*, *IL1f7*, *IL3*, *IL7Ra*, *IL17A*, *IL18*, *IL18R*, *IL18RAP*, *IL28B*, *IL15RA*, *IL15*, *IL9*, *IL10*, *IL12A*, *IL12B*, and *IL13* genes and the risk of CAD. In addition, a meta-analysis was conducted to elucidate the genetic contribution of the positive locus to the risk of CAD.

## Materials and methods

### *Ethics statement*

This case-control study protocol was reviewed and approved by Ethical Committee of ZhongDa Hospital Affiliated to Southeast University and the clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. After given the written informed consent to participate, all the subjects were interviewed to collect information on demographic data.

### *Sample collection*

A total of 826 subjects with CAD [males: 592 (71.67%); age: 67.27 $\pm$ 10.26 years] were consecutively recruited from Zhongda Hospital Affiliated to Southeast University (Nanjing, China) from July 2005 to December 2008. 1153 controls [males: 510 (44.23%); age: 59.13 $\pm$ 10.51 years] were non-CAD subjects recruited from Zhenjiang who participated the physical examination, China. All patients underwent coronary angiography (CAG) and the results were judged by two or three experienced cardiologists. CAD cases were patients having the presence of at least one significant coronary artery stenosis of  $\geq$  50% luminal diameter on coronary angiography. Non-CAD controls had no symptoms of angina or possible myocardial infarction, and had no history of CAD. Those with congenital heart disease, cardiomyopathy, severe liver or kidney disease, and malignant tumors were excluded from this study.

### *SNP genotyping*

Blood samples were collected from each subject using vacutainers with ethylenediamine tetra-acetic acid (EDTA). Genomic DNA was extracted from whole blood using the Qiagen DNA Blood Mini Kit (Qiagen, Berlin, Germany). SNPs genotyping were performed utilizing a custom-by-design 48-Plex SNPscanTM Kit (Genesky Biotechnologies Inc., Shanghai, China) as previously described [25]. Repeated analyses were conducted for 4% of randomly selected samples with high DNA quality.

### *Systematic review and meta-analysis*

Literature searches of the PubMed, EMBASE, Web of Science, Chinese National Knowledge

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Infrastructure (CNKI), and WanFang Database were conducted to identify eligible studies, with the last search update on March 31, 2015. Taking the keywords 'coronary artery disease', 'CAD', 'coronary heart disease', 'CHD', 'IL10', 'IL28B', 'IL1f7', 'polymorphism', 'genotype', 'variation', 'SNP', 'variant' and 'allele' as the search terms, without any language, country or publication status restrictions applied. Additional relevant publications were identified by reviewing the reference lists of retrieved articles. Studies selected for further meta-analysis must meet the following inclusion criteria: (1) reports about associations between IL10 rs1800872 G>T, IL28B rs8099917 T>G, or IL1f7 rs3811047 G>A polymorphism and risk of CAD; (2) case-control design; (3) sufficient data provided to assess odds ratios (ORs) and the corresponding confidence intervals (CIs). Exclusion criteria: (1) duplicate data; (2) meeting abstract, comment, review or editorial. Data was extracted from all included studies by two independent investigators based on a standard protocol, and consensus data were established through discussion. In the current meta-analysis, we collect the first author's name, publication year, country and ethnicity of population, mean age of case and controls, source of controls, diagnostic criteria, genotyping methods, number of cases and controls, and allelic and genotypic frequency and their distribution in cases and controls. Inter-researcher disagreement was solved by discussion.

### Statistical analyses

For our case-control study, quantitative variables are uniformly expressed as mean  $\pm$  standard deviation (SD), and the categorical variables are presented as absolute frequencies or percentages. The differences of the demographic characteristics between the cases and controls were evaluated using the  $\chi^2$  test (for categorical variables) and Student's *t* test (for quantitative variables). The associations of 28 SNPs genotypes with CAD risk were assessed by calculating odds ratios (ORs) utilizing logistic regression analyses for crude ORs and adjusted ORs (adjusted for age and sex). All the 28 SNPs were tested for conformity to Hardy-Weinberg expectations by a goodness-of-fit  $\chi^2$  test among controls subjects. The power of this study was estimated using the Power and Sample Size Calculation software (v3.0.43). All

statistical analyses were performed using the STATA 12.0 software (StataCorp, College Station, TX, USA), and the *P* value less than 0.05 in two sides was accepted as statistically significant.

In the following meta-analysis, we initially assessed HWE for each study by  $\chi^2$  test in the controls groups. The strength of associations between SNPs and CAD was evaluated by pooled OR with its corresponding 95% CI. The pooled ORs were calculated for allelic model, dominant model and recessive model. Statistical heterogeneity between eligible studies was evaluated by using the Cochran's *Q* statistic and *I*<sup>2</sup> test [26, 27]. *P* < 0.1 and *I*<sup>2</sup> exceeding 50% indicated substantial heterogeneity across studies, then a random effects model was chosen to perform meta-analysis, otherwise, the fixed-effects model was selected. Stratification analyses by ethnicity were performed to evaluate the ethnic effects. Additionally, a subgroup analysis of controls with different selection criteria (normal angiography and symptom investigation) was also performed. The stability of the results was evaluated using a sensitivity analysis in which each study was deleted each time to determine if any studies significantly affected the pooled OR. Begg's funnel plot and Egger's regression test were used to search for publication bias [28, 29]. The fail-safe number ( $N_{fs}$ ) set at a significance of 0.05 was also calculated to inspect publication bias, according to the formula  $N_{fs,0.05} = (\sum Z/1.64)^2 - k$ , where *k* is the number of studies included. If the  $N_{fs}$  was less than the number of observed studies for a polymorphism, we deemed that there exists a significant publication bias for the meta-result.

### Results

#### *Characteristics of the study population and the information of 28 SNPs in interleukin genes*

Mean age of CAD patients was higher than that of control, and the proportion of female in the CAD group was lower than in the control group (all comparison *P* < 0.05). **Table 1** shows the primary information for 28 Genotyped SNPs. All the polymorphisms passed the Hardy-Weinberg equilibrium (HWE) test for the distribution of genotypes in non-CAD controls (*P*>0.05), indicating our sampling method was random. The genotyping success rate of overall SNP was

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**Table 1.** Primary information for 28 Genotyped SNPs

Genotyped SNPs	Chr <sup>a</sup>	Regulome DB Score <sup>b</sup>	Location	TFBS <sup>c</sup>	Functional Consequence	MAF <sup>d</sup> for Chinese in database	MAF in our controls (n = 826)	P-value for HWE <sup>e</sup> test in our controls	Genotyping value (%)
<i>IL1A: rs1800587 C&gt;T</i>	2	5	5'-Flanking	Y	Utr variant 5 prime	0.047	0.091	0.612	98.8
<i>IL1B: rs16944 G&gt;A</i>	2	1f	5'-Flanking	Y	Upstream variant 2 KB	0.547	0.532	0.058	98.6
<i>IL1f7: rs3811047 G&gt;A</i>	2	No Data	nonsynon_exon 2	--	Intron variant	0.209	0.189	0.972	99.4
<i>IL3: rs40401 C&gt;T</i>	5	1f	5'-Flanking	--	Missense	0.581	0.504	0.977	99.2
<i>IL3: rs2073506 G&gt;A</i>	5	5	5'-Flanking	Y	Upstream variant 2 KB	0.186	0.160	0.595	99.5
<i>IL7Ra: rs6897932 C&gt;T</i>	5	5	Exon 6	--	Intron variant	0.198	0.145	0.502	99.1
<i>IL17A: rs2275913 G&gt;A</i>	6	No Data	5'-Flanking	Y	Upstream variant 2 KB	0.465	0.466	0.174	99.3
<i>IL17A: rs3819024 A&gt;G</i>	6	No Data	5'-Flanking	Y	upstream variant 2 KB	0.477	0.479	0.405	99.2
<i>IL17A: rs3819025 G&gt;A</i>	6	4	Intron 1	Y	Intron variant	0.198	0.156	0.471	99.2
<i>IL17A: rs4711998 A&gt;G</i>	6	No data	5'-Flanking	Y	Upstream variant 2 KB	0.756	0.741	0.572	97.6
<i>IL17A: rs8193036 C&gt;T</i>	6	No data	5'-Flanking	Y	Upstream variant 2 KB	0.640	0.727	0.211	95.6
<i>IL17A: rs8193037 G&gt;A</i>	6	No Data	5'-Flanking	Y	Upstream variant 2 KB	0.058	0.116	0.193	99.5
<i>IL18: rs360719 A&gt;G</i>	11	2 <sup>b</sup>	5'-Flanking	Y	Upstream variant 2 KB	0.142	0.128	0.521	98.5
<i>IL18R: rs13015714 T&gt;G</i>	2	5	5'-Flanking	--	Upstream variant 2 KB	0.453	0.486	0.498	99.3
<i>IL18RAP: rs917997 T&gt;C</i>	2	No Data	3'-Flanking	--	Downstream gene variant	0.465	0.495	0.884	98.0
<i>IL28B: rs8099917 T&gt;G</i>	19	4	5'-Flanking	--	Upstream gene variant	0.035	0.056	0.375	99.3
<i>IL15RA: rs2228059 A&gt;C</i>	10	4	Nonsynon_exon 4	--	Missense	0.547	0.589	0.612	99.4
<i>IL15: rs10519612 A&gt;C</i>	4	6	Intron 7	--	Intron variant	0.474	0.425	0.278	99.0
<i>IL15: rs10519613 C&gt;A</i>	4	No Data	3'-UTR_exon8	--	Utr variant 3 prime	0.500	0.423	0.156	99.4
<i>IL15: rs17007695 T&gt;C</i>	4	4	3'-Flanking	--	Intergenic variant	0.477	0.412	0.975	99.3
<i>IL15: rs17015014 G&gt;C</i>	4	3 <sup>a</sup>	3'-Flanking	--	Regulatory region variant	0.522	0.476	0.853	99.5
<i>IL15: rs35964658 A&gt;G</i>	4	5	3'-Flanking	--	Intergenic variant	0.488	0.447	0.222	99.4
<i>IL9: rs31563 C&gt;T</i>	5	5	5'-Flanking	Y	Intron variant	0.116	0.131	0.936	96.7
<i>IL9: rs31564 G&gt;T</i>	5	5	Intron 3	--	Intron variant	0.581	0.549	0.724	99.5
<i>IL10: rs1800872 G&gt;T</i>	1	5	5'-Flanking	Y	Upstream variant 2 KB	0.733	0.691	0.226	98.5
<i>IL12A: rs2243115 T&gt;G</i>	3	4	5'-Flanking	--	Upstream variant 2 KB	0.093	0.091	0.364	99.4
<i>IL12B: rs3212227 T&gt;G</i>	5	No Data	3'-UTR	--	Utr variant 3 prime	0.430	0.441	0.467	98.9
<i>IL13: rs1800925 C&gt;T</i>	5	2 <sup>b</sup>	5'-Flanking	Y	Upstream variant 2 KB	0.167	0.156	0.598	99.3

<sup>a</sup>Chr, chromosome; <sup>b</sup><http://www.regulomedb.org/>; <sup>c</sup>TFBS, transcription factor binding site (<http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm>); <sup>d</sup>MAF, minor allele frequency, *IL18* rs360719 A/G and *IL13* rs1800925 C/T MAF is in CHB+JPT population; <sup>e</sup>HWE, Hardy-Weinberg equilibrium.

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**Table 2.** Main effects of SNPs on CAD risk

Genotyped SNPs	Genotyping		P	AB vs. AA OR (95% CI); P	BB vs. AA OR (95% CI); P
	Case (n = 826) (AA/AB/BB)	Control (n = 1153) (AA/AB/BB)			
<i>IL1A</i> : rs1800587 C>T	680/133/5	939/191/8	0.923	0.96 (0.75-1.23); 0.751	0.86 (0.28-2.65); 0.797
<i>IL1B</i> : rs16944 G>A	232/393/189	306/598/233	0.149	0.87 (0.70-1.07); 0.188	1.07 (0.83-1.38); 0.606
<i>IL1f7</i> : rs3811047 G>A	583/209/32	752/350/41	0.039	<b>0.77 (0.63-0.94); 0.012</b>	1.01 (0.63-1.62); 0.978
<i>IL3</i> : rs40401 C>T	215/406/204	294/560/284	0.990	0.99 (0.80-1.23); 0.938	0.98 (0.76-1.26); 0.889
<i>IL3</i> : rs2073506 G>A	562/249/15	810/301/32	0.083	1.19 (0.98-1.46); 0.084	0.68 (0.36-1.26); 0.217
<i>IL7Ra</i> : rs6897932 C>T	613/191/20	829/287/21	0.418	0.90 (0.73-1.11); 0.327	1.29 (0.69-2.40); 0.425
<i>IL17A</i> : rs2275913 G>A	226/437/161	337/545/259	0.060	1.20 (0.97-1.48); 0.096	0.93 (0.72-1.20); 0.566
<i>IL17A</i> : rs3819024 A>G	211/436/177	316/555/269	0.180	1.18 (0.95-1.46); 0.138	0.99 (0.76-1.27); 0.911
<i>IL17A</i> : rs3819025 G>A	567/234/22	815/294/31	0.427	1.14 (0.93-1.40); 0.193	1.02 (0.58-1.78); 0.944
<i>IL17A</i> : rs4711998 A>G	442/288/70	618/441/72	0.092	0.91 (0.75-1.11); 0.354	1.36 (0.96-1.93); 0.086
<i>IL17A</i> : rs8193036 C>T	409/294/70	599/428/92	0.815	1.01 (0.83-1.22); 0.952	1.11 (0.80-1.56); 0.527
<i>IL17A</i> : rs8193037 G>A	653/160/13	897/226/20	0.928	0.97 (0.78-1.22); 0.809	0.89 (0.44-1.81); 0.753
<i>IL18</i> : rs360719 A>G	608/202/12	855/256/16	0.627	1.11 (0.90-1.37); 0.336	1.05 (0.50-2.25); 0.890
<i>IL18R</i> : rs13015714 T>G	201/415/209	295/581/264	0.492	1.05 (0.84-1.31); 0.673	1.16 (0.90-1.50); 0.249
<i>IL18RAP</i> : rs917997 T>C	213/402/195	278/562/289	0.618	0.93 (0.75-1.16); 0.540	0.88 (0.68-1.14); 0.328
<i>IL28B</i> : rs8099917 T>G	758/63/2	1016/124/2	0.055	<b>0.68 (0.50-0.94); 0.018</b>	1.34 (0.19-9.54); 0.770
<i>IL15RA</i> : rs2228059 A>C	296/396/133	401/545/197	0.798	0.98 (0.81-1.20); 0.876	0.91 (0.70-1.19); 0.511
<i>IL15</i> : rs10519612 A>C	280/395/149	367/573/196	0.549	0.90 (0.74-1.11); 0.324	1.00 (0.77-1.30); 0.979
<i>IL15</i> : rs10519613 C>A	281/394/150	368/581/193	0.391	0.89 (0.73-1.09); 0.248	1.02 (0.78-1.33); 0.896
<i>IL15</i> : rs17007695 T>C	269/404/150	394/554/194	0.529	1.07 (0.87-1.31); 0.521	1.13 (0.87-1.47); 0.355
<i>IL15</i> : rs17015014 G>C	225/400/201	316/567/260	0.712	0.99 (0.80-1.23); 0.932	1.09 (0.84-1.40); 0.521
<i>IL15</i> : rs35964658 A>G	259/412/154	339/585/218	0.718	0.92 (0.75-1.13); 0.436	0.92 (0.71-1.20); 0.558
<i>IL9</i> : rs31563C>T	602/173/12	851/257/19	0.873	0.95 (0.76-1.18); 0.657	0.89 (0.43-1.85); 0.761
<i>IL9</i> : rs31564 G>T	247/415/164	348/560/235	0.854	1.04 (0.85-1.28); 0.682	0.98 (0.76-1.27); 0.898
<i>IL10</i> : rs1800872 G>T	95/381/343	99/500/532	0.027	0.79 (0.58-1.08); 0.147	<b>0.67 (0.49-0.92); 0.013</b>
<i>IL12A</i> : rs2243115 T>G	688/129/8	947/184/12	0.946	0.97 (0.75-1.23); 0.776	0.91 (0.37-2.26); 0.852
<i>IL12B</i> : rs3212227 T>G	268/395/155	362/550/228	0.811	0.97 (0.79-1.19); 0.771	0.92 (0.71-1.19); 0.517
<i>IL13</i> : rs1800925 C>T	579/223/24	814/295/30	0.789	1.06 (0.87-1.30); 0.559	1.12 (0.65-1.94); 0.674

AA/AB/BB means homozygote, heterozygote and mutated homozygote; Bold values are statistically significant (P < 0.05).

high, ranging from 95.6% to 99.5%. The concordance rates of repeated analyses were 100%. Minor allele frequency (MAF) of all SNPs in our controls range from 0.056-0.741 and was similar to MAF for Chinese in database (<http://www.ncbi.nlm.nih.gov/snp/>).

### Associations between 28 SNPs and the risk of CAD

No significant differences were observed in the distributions of the genotypes between the CAD group and the control group (all P>0.05), except for *IL1f7* rs3811047 G>A and *IL10* rs1800872 G>T (**Table 2**). We further examined the relationship between the genetic variants and CAD risk, and found that subjects carrying *IL1f7* rs3811047 GA/AA, *IL28B* rs8099917 GT/GG and *IL10* rs1800872 TT/GT genotypes had the less risk of CAD in dominant model (rs3811047, GA+AA vs. GG: adjusted OR = 0.77,

95% CI = 0.62-0.96, P = 0.021; rs8099917, GT+GG vs. TT: adjusted OR = 0.69, 95% CI = 0.49-0.98, P = 0.040 and rs1800872, TT+GT vs. GG: adjusted OR = 0.71, 95% CI = 0.51-0.98, P = 0.040) (**Table 3**). But after the Bonferroni correction (0.05/28 = 0.0018), the three loci showed no significant association with CAD risk. No significant associations between the other 25 SNPs with CAD were found in this case-control study. In the dominant model, stratification analysis by gender and age groups (≤60 or >60 years) also showed that the male carrying rs3811047 GA/AA genotypes had a 27% decreased risk of CAD (OR = 0.73, 95% CI = 0.56-0.93, P = 0.013), and elder persons carrying rs8099917 GT/GG genotypes had a 35% decreased risk of CAD (OR = 0.65, 95% CI = 0.44-0.95, P = 0.026). Furthermore, the elder female CAD patients carrying rs1800872 TT genotype had a 42% decreased

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**Table 3.** Logistic regression analyses of associations between *IL1f7*: rs3811047 G>A, *IL28B*: rs8099917 T>G, *IL10* rs1800872 G>T and risk of CAD

Genotype	Cases (n = 826) <sup>a</sup>		Controls (n = 1153)		Crude OR (95% CI)	P	Adjusted OR <sup>b</sup> (95% CI)	P
	n	%	n	%				
<i>IL1f7</i> :								
rs3811047 G>A								
GG	583	70.75	753	65.79	1.00	-	1.00	-
GA	209	25.37	350	30.62	<b>0.77 (0.63-0.94)</b>	<b>0.012</b>	<b>0.75 (0.60-0.94)</b>	<b>0.012</b>
AA	32	3.88	41	3.59	1.01 (0.63-1.62)	0.978	1.01 (0.59-1.73)	0.982
GA+AA	241	29.25	391	34.21	<b>0.80 (0.66-0.96)</b>	<b>0.020</b>	<b>0.77 (0.62-0.96)</b>	<b>0.021</b>
GG+GA	792	96.12	1103	96.41	1.00	-	1.00	-
AA	32	3.88	41	3.59	1.09 (0.68-1.74)	0.732	1.10 (0.64-1.87)	0.74
G allele	1375	83.43	1856	81.12	1.00	-	-	-
A allele	273	16.57	432	18.88	0.85 (0.72-1.01)	0.062	-	-
<i>IL28B</i> :								
rs8099917 T>G								
TT	758	92.10	1016	88.97	1.00	-	1.00	-
GT	63	7.66	124	10.86	<b>0.68 (0.50-0.94)</b>	<b>0.018</b>	<b>0.67 (0.47-0.96)</b>	<b>0.029</b>
GG	2	0.24	2	0.17	1.34 (0.19-9.54)	0.770	2.12 (0.28-15.93)	0.465
GT+GG	65	7.9	126	11.03	<b>0.69 (0.51-0.95)</b>	<b>0.021</b>	<b>0.69 (0.49-0.98)</b>	<b>0.040</b>
TT+GT	821	99.76	1140	99.83	1.00	-	1.00	-
GG	2	0.24	2	0.17	1.39 (0.20-9.88)	0.743	2.20 (0.29-16.49)	0.444
T allele	1579	95.93	2156	94.40	1.00	-	-	-
G allele	67	4.07	128	5.60	<b>0.71 (0.53-0.97)</b>	<b>0.030</b>	-	-
<i>IL10</i> :								
rs1800872 G>T								
GG	95	11.60	99	8.75	1.00	-	1.00	-
GT	381	46.52	500	44.21	0.79 (0.58-1.08)	0.147	0.77 (0.54-1.09)	0.146
TT	343	41.88	532	47.04	<b>0.67 (0.49-0.92)</b>	<b>0.013</b>	<b>0.64 (0.45-0.91)</b>	<b>0.013</b>
GT+TT	724	88.40	1032	91.25	<b>0.73 (0.54-0.98)</b>	<b>0.039</b>	<b>0.71 (0.51-0.98)</b>	<b>0.040</b>
GG+GT	476	58.12	599	52.96	1.00	-	1.00	-
TT	343	41.88	532	47.04	0.81 (0.68-0.97)	0.024	0.79 (0.65-0.97)	0.026
G allele	571	34.86	698	30.86	1.00	-	-	-
T allele	1067	65.14	1564	69.14	<b>0.83 (0.73-0.95)</b>	<b>0.009</b>	-	-

<sup>a</sup>The genotyping was successful in 824 (99.8%) CAD cases, and 1144 (99.2%) controls for *IL1f7* rs3811047 G>A, 823 (99.6%) CAD cases, and 1142 (99.0%) controls for *IL28B* rs8099917 T>G, 819 (99.2%) CAD cases, and 1131 (98.1%) controls for *IL10* rs1800872 G>T; <sup>b</sup>Adjusted for age and sex. Bold values are statistically significant (P < 0.05).

risk of CAD (OR = 0.58, 95% CI 0.35-0.97, P = 0.038) (**Table 4**).

### Meta-analysis on *IL10* rs1800872 G>T polymorphism and CAD risk

A flow diagram describing the literature search and study identification was shown in **Figure 1**. Nine case-control studies on the *IL10* rs1800872 G>T polymorphism and CAD risk were collected including 3038 patients and

2732 controls [30-37]. Of these studies, 3 studies were carried out in Caucasian populations (1664 cases, 2017 controls) [31, 36, 37], and other 6 studies, including our study, were in Asian populations (1374 cases, 715 controls) [30, 32-35]. The general characteristics of all eligible studies were summarized in **Table 5**. MAF of T allele in the controls was ranged from 0.200 in Caucasians to 0.736 in Asians. The genotypes distributions of the control groups conformed to the HWE except the reports by

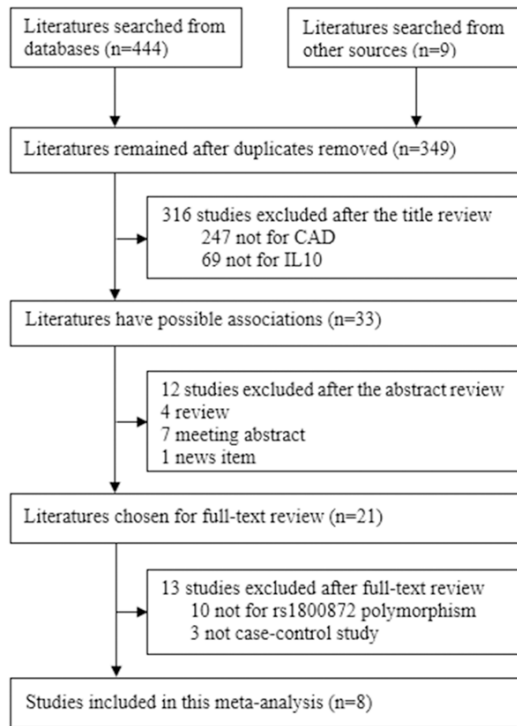
## IL10 gene polymorphism and risk of CAD

**Table 4.** Multivariate associations of the *IL1f7* rs3811047 G>A, *IL28B* rs8099917 T>G and *IL10* rs1800872 G>T polymorphisms with the CAD risk by further stratification for sex and age

Variable	<i>IL1f7</i> : rs3811047 G>A (case/control) <sup>a</sup>				Adjusted OR <sup>b</sup> (95% CI); P				
	GG	GA	AA	GA+AA	GG	GA	AA	GA+AA	AA vs. (GA+GG)
Sex									
Male	414/319	151/169	25/18	176/187	1.00	<b>0.69 (0.53-0.90); P: 0.006</b>	1.07 (0.57-2.00); p: 0.831	<b>0.73 (0.56-0.93); P: 0.013</b>	1.20 (0.65-2.23); p: 0.564
Female	168/433	58/181	6/23	64/204	1.00	0.83 (0.58-1.17); P: 0.278	0.67 (0.27-1.68); P: 0.396	0.81 (0.58-1.13); P: 0.210	0.71 (0.28-1.76); P: 0.459
Age									
< 60	125/368	42/177	9/23	51/200	1.00	0.70 (0.47-1.03); P: 0.074	1.15 (0.52-2.56); P: 0.728	0.75 (0.52-1.09); P: 0.127	1.28 (0.58-2.81); P: 0.544
≥ 60	458/384	167/173	23/18	190/191	1.00	0.81 (0.63-1.04); P: 0.100	1.07 (0.57-2.01); P: 0.831	0.83 (0.65-1.06); P: 0.142	1.14 (0.61-2.13); P: 0.685
<i>IL28B</i> : rs8099917 T>G (case/control) <sup>a</sup>									
	TT	GT	GG	GT+GG	TT	GT	GG	GT+GG	GG vs. (GT+TT)
Sex									
Male	541/450	46/55	2/1	48/56	1.00	0.70 (0.46-1.05); P: 0.084	1.66 (0.15-18.41); P: 0.678	0.71 (0.48-1.07); P: 0.102	1.72 (0.16-19.03); P: 0.658
Female	215/566	17/69	0/1	17/70	1.00	0.65 (0.37-1.13); P: 0.125	1 EMPTY	0.64 (0.37-1.11); P: 0.113	1 EMPTY
Age									
< 60	161/507	13/59	2/1	15/60	1.00	0.69 (0.37-1.30); P: 0.253	6.30 (0.57-69.91); P: 0.134	0.79 (0.44-1.42); P: 0.429	6.51 (0.59-72.18); P: 0.127
≥ 60	597/509	50/65	0/1	50/66	1.00	<b>0.66 (0.45-0.97); P: 0.033</b>	1 EMPTY	<b>0.65 (0.44-0.95); P: 0.026</b>	1 EMPTY
<i>IL10</i> : rs1800872 G>T (case/control) <sup>a</sup>									
	GG	GT	TT	GT+TT	GG	GT	TT	GT+TT	TT vs. (GT+GG)
Sex									
Male	66/43	266/218	254/241	520/459	1.00	0.79 (0.52-1.21); P: 0.289	0.69 (0.45-1.05); P: 0.081	0.74 (0.49-1.11); P: 0.141	0.83 (0.65-1.05); P: 0.124
Female	29/56	114/282	88/291	202/573	1.00	0.78 (0.47-1.28); P: 0.330	<b>0.58 (0.35-0.97); P: 0.038</b>	0.68 (0.42-1.10); P: 0.113	<b>0.71 (0.53-0.97); P: 0.033</b>
Age									
< 60	19/48	79/253	79/258	158/511	1.00	0.79 (0.44-1.42); P: 0.429	0.77 (0.43-1.39); P: 0.392	0.78 (0.45-1.37); P: 0.388	0.94 (0.67-1.32); P: 0.723
≥ 60	76/51	302/247	264/274	566/521	1.00	0.82 (0.55-1.21); P: 0.323	<b>0.65 (0.44-0.96); P: 0.030</b>	0.73 (0.50-1.06); P: 0.098	<b>0.76 (0.61-0.95); P: 0.018</b>

<sup>a</sup>The genotyping was successful in 824 (99.8%) CAD cases, and 1144 (99.2%) controls for *IL1f7* rs3811047 G>A, 823 (99.6%) CAD cases, and 1142 (99.0%) controls for *IL28B* rs8099917 T>G, 819 (99.2%) CAD cases, and 1131 (98.1%) controls for *IL10* rs1800872 G>T; <sup>b</sup>Adjusted for age and sex (besides stratified actors accordingly) in a logistic regression model. Bold values are statistically significant (P < 0.05).

## IL10 gene polymorphism and risk of CAD



**Figure 1.** Flow diagram of the search strategy and study selection. The terms “N” in the boxes represent the number of corresponding studies.

Afzal et al. and Zhou et al. [32, 35], which were also considered for the meta-analysis, but excluded in the sensitivity analysis. The random effects model was analyzed for all the genetic models because of the heterogeneity of the studies. Overall, the pooled estimates revealed that the *IL10* rs1800872 G>T polymorphism was not significantly associated with CAD risk in all genetic models (T vs. G: OR = 1.07, 95% CI 0.88-1.30, P = 0.510; TT+TG vs. GG: OR = 1.16, 95% CI 0.88-1.54, P = 0.285; TT vs. TG+GG: OR = 0.96, 95% CI 0.73-1.27, P = 0.777) (**Figure 2**). And this no significant effect was also found in the subgroups by ethnicity and control selection criteria (**Table 6**). Sensitivity analysis was conducted to evaluate the weight of single study on the pooled OR. The results were all statistically similar when sequential remove each eligible study. For publication bias, both the shape of the Begg’s funnel plot and Egger’s test indicated no publication bias was found in this meta-analysis (TT+TG vs. GG:  $t = 1.07$ , P = 0.319; **Figure 3**). The  $N_{fs0.05}$  of this study was 86, which is greater than the number of studies included in this meta-analysis, also indicating a low probability of publica-

tion bias. For the *IL1f7* rs3811047 G>A or *IL28B* rs8099917 T>G genetic variants and CAD risk, few studies were concerned about them, so we did not conduct the meta-analysis on these two loci.

### Discussion

CAD is one of the chronic inflammatory diseases [38]. Interleukins, a group of cytokines, were recognized as crucial agents involved in the inflammatory response that contributes to atherosclerosis. In this study, we investigated the relationship between the 28 SNPs in *IL1A*, *IL1B*, *IL1f7*, *IL3*, *IL7Ra*, *IL17A*, *IL18*, *IL18R*, *IL18RAP*, *IL28B*, *IL15RA*, *IL15*, *IL9*, *IL10*, *IL12A*, *IL12B*, and *IL13* genes and the risk of CAD. The multivariable logistic analysis showed that *IL1f7* rs3811047 G>A, *IL28B* rs8099917 T>G and *IL10* rs1800872 G>T genetic variants were significantly associated with the decreased risk of CAD. In addition, through gender/age-stratified comparison, male carrying rs3811047 GA/AA genotypes had a 27% decreased risk of CAD, and elder persons carrying rs8099917 GT/GG genotypes had a 35% decreased risk of CAD. Furthermore, the elder female CAD patients carrying rs1800872 TT genotype had a 42% decreased risk of CAD. No significant association was found for the relationship between the other 25 SNPs and CAD in the present study of a China case-control sample. While a meta-analysis indicated that there is no significant association between *IL10* rs1800872 G>T polymorphism and CAD risk.

*IL1f7*, namely *IL37*, is a newly defined member of the *IL-1* cytokine family and play a significant regulatory role in regulating inflammatory response [7]. *IL-37* was expressed in the foam-like cells of atherosclerotic coronary and carotid artery plaques, and it may exert protective effects on atherosclerosis-related diseases by suppressing the production of pro-inflammatory cytokines and the activation of macrophage [9, 10]. *IL1f7* gene SNP rs3811047 G>A was a nonsynonymous variant. Recently, epidemiology studies found that *IL1f7* rs3811047 AA genotype was significantly associated with a decreased risk of gastric cardiac adenocarcinoma, and *IL1f7* rs3811047 A allele may be a protective factor for rheumatoid arthritis [39, 40]. In this case-control study, we also found that subjects with the *IL1f7* rs3811047 (GA+AA) variant genotypes had 0.23-fold decreased risk of CAD.



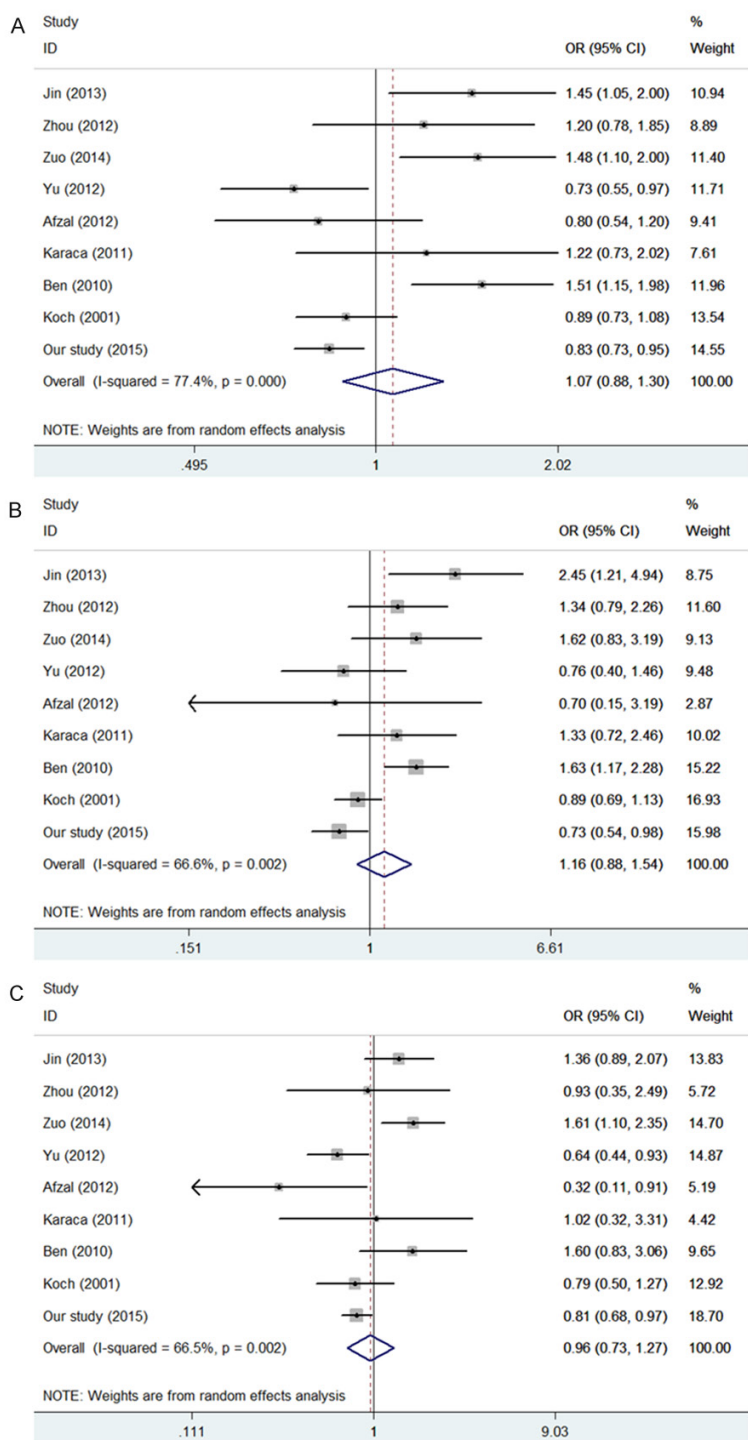
## IL10 gene polymorphism and risk of CAD

**Table 5.** Main characteristics of the studies included in the meta-analysis

Study name	Country	Ethnicity	Enrollment criteria		Genotyping method	Case			Control			MAF	Case/Control			HWE
			Case	Control		N	Age	Male (%)	N	Age	Male (%)		TT	GT	GG	
Jin 2013	China	Asian	Angiography confirmed CAD ( $\geq$ 50% stenosis)	Normal angiography	MALDI-TOF MS	249	65.85 $\pm$ 9.85	70.3	132	63.60 $\pm$ 9.05	61.4	0.659	134/61	99/52	16/19	0.156
Karaca 2011	Turkey	Caucasian	Angiography confirmed CHD ( $\geq$ 50% stenosis)	Symptom investigation	PCR-RFLP	86	43.36 $\pm$ 4.93	69.8	88	47.07 $\pm$ 8.14	71.6	0.205	6/6	29/24	51/58	0.129
Zhou 2012	China	Asian	Angiography confirmed CAD ( $\geq$ 50% stenosis)	Normal angiography	PCR-RFLP	118	46.42 $\pm$ 4.74	68.6	124	47.54 $\pm$ 4.56	66.1	0.206	8/9	40/33	70/82	0.039
Zuo 2014	China	Asian	Angiography confirmed CHD	Symptom investigation	TaqMan	212	< 65 102 $\geq$ 65 116	77.4	218	< 65 108 $\geq$ 65 104	78.9	0.683	126/104	71/90	15/24	0.499
Yu 2012	Korean	Asian	Angiography Confirmed IHD	Symptom investigation	pyrosequencing	173	61.64 $\pm$ 9.83	67.1	313	61.37 $\pm$ 12.58	37.4	0.736	76/172	80/117	17/24	0.511
Afzal 2012	Pakistan	Asian	CAD	Symptom investigation	ARMS-PCR	93	NA	NA	99	NA	NA	0.561	5/15	84/81	4/3	< 0.001
Ben 2010	Tunisia	Caucasian	Angiography confirmed CAD ( $\geq$ 50% stenosis)	Symptom investigation	PCR-ASA	291	56.7 $\pm$ 12.2	75.6	291	56.3 $\pm$ 12.5	75.6	0.200	25/16	109/83	156/188	0.099
Koch 2001	Germany	Caucasian	Angiography confirmed CAD ( $\geq$ 50% stenosis)	Normal angiography	AS-PCR	998	64.1 $\pm$ 10.2	75.9	340	63.4 $\pm$ 10.3	75.3	0.282	64/27	390/138	544/175	0.977
This study 2015	China	Asian	Angiography confirmed CAD ( $\geq$ 50% stenosis)	Symptom investigation	PCR	819	67.27 $\pm$ 10.26	71.67	1131	59.13 $\pm$ 10.51	44.23	0.691	343/532	381/500	95/99	0.226

NA, not available; CAD, coronary artery disease; CHD, coronary heart disease; IHD, ischemic heart disease; PCR, polymerase chain reaction; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR-ASA, polymerase chain reaction-allele specific amplification; ARMS-PCR, amplification refractory mutation system-polymerase chain reaction; AS-PCR, allele specific- polymerase chain reaction; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium.

## IL10 gene polymorphism and risk of CAD



**Figure 2.** The ORs of *IL10* rs1800872 G>T polymorphism and CAD risk in different genetic models (A: T vs. G; B: TT+TG vs. GG; C: TT vs. TG+GG). The random effects model was used to pool the effect sizes for all the genetic models.

*IL-28B* (IFN $\lambda$ 3) is a cytokine mainly released by macrophages and dendritic cells, and it plays a crucial role in antiviral immunity. *IL28B* was

associated with hepatic lobular inflammation and fibrosis, and thus it could also be linked to the severity of systemic in particular cardiovascular alterations in CAD patients [13]. Rs8099917 T>G is located 8.9 kb upstream of the start codon of *IL28B* [41]. Accumulating evidence suggests that *IL28B* rs8099917 T>G appear to be strongly related with spontaneous HCV clearance and sustained virological response in chronic HCV infection patients who undergoing combination therapy [42-44]. In this study, we found that humans who carry the variant G allele have a 29% decreased risk of CAD compared with subjects who have the T allele. The phenomenon may be related to the SNP rs8099917-G allele which modulates the activity of *IL28B*. To our knowledge, this investigation is the first clinical study that explore the association between *IL1f7* rs3811047 G>A or *IL28B* rs8099917 T>G polymorphisms and CAD risk.

*IL-10* is a pleiotropic cytokine inhibits monocyte/macrophage function during inflammation through down-regulating the production of pro-inflammatory cytokines. *IL10* gene is located on human chromosome 1, has five exons and mapped to the junction between 1q31 and 1q32. *IL10* was shown to protect endothelial function after an acute inflammatory stimulus by limiting increases in superoxide generation within the vascular wall, which implies a key role in progression of CAD [45]. Previous study has dem-

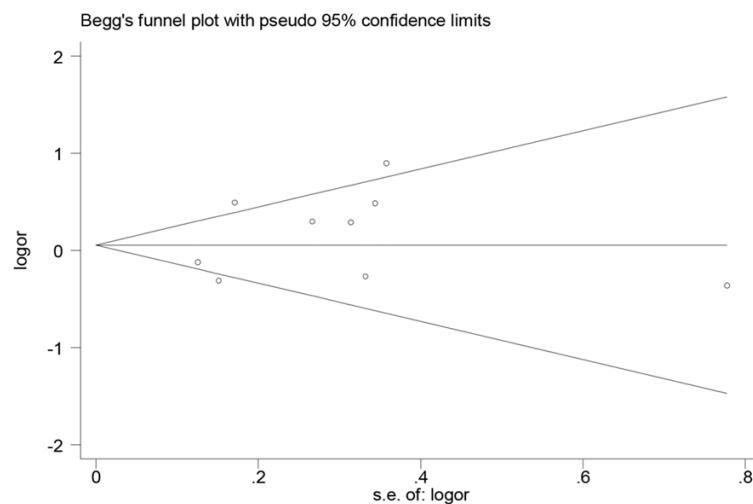
onstrated that rs18000872 polymorphism in the *IL-10* gene promoter region can influence the *IL-10* expression [46]. Recently, the rela-

## IL10 gene polymorphism and risk of CAD

**Table 6.** Summary ORs of the relationship between *IL10* rs1800872 G>T polymorphism and CAD risk by ethnicity and Control selection

Variables	N <sup>a</sup>	T vs. G			TT+TG vs. GG			TT vs. TG+GG		
		OR (95% CI)	P <sup>b</sup>	I <sup>2</sup> (%)	OR (95% CI)	P <sup>b</sup>	I <sup>2</sup> (%)	OR (95% CI)	P <sup>b</sup>	I <sup>2</sup> (%)
Ethnicity										
Asian	6	1.03 (0.80-1.32)	< 0.001	78.8	1.14 (0.75-1.75)	0.012	65.7	0.92 (0.64-1.31)	0.001	75.7
Caucasian	3	1.16 (0.79-1.70)	0.008	79.3	1.22 (0.78-1.90)	0.013	76.8	1.02 (0.71-1.47)	0.231	31.8
Control selection										
Normal angiography	3	1.13 (0.81-1.57)	0.032	71.0	1.32 (0.76-2.30)	0.016	75.8	1.06 (0.78-1.43)	0.243	29.4
Symptom investigation	6	1.04 (0.79-1.36)	< 0.001	82.0	1.10 (0.75-1.63)	0.008	68.1	0.93 (0.63-1.36)	0.001	74.9

<sup>a</sup>Number of comparisons; <sup>b</sup>P value for heterogeneity.



**Figure 3.** Funnel plot of *IL10* rs1800872 G>T polymorphism and CAD risk for publication bias (TT+TG vs. GG). Each circle represents a separate study for the indicated association. logor, natural logarithm of OR; s.e., standard error.

relationship between *IL10* rs1800872 G>T polymorphism and CAD risk was investigated among different ethnic groups. The association was firstly reported by Koch et al., which suggest that *IL10* rs1800872 G>T polymorphism had a negative association with the risk of CAD in Caucasians. However, Ben and colleagues discovered that rs1800872 T allele was a risk genetic factor to contribute to CAD, but Yu's study suggested that rs1800872 G allele was associated with an increased risk for CAD in Asians. The discrepancy between these results may be due to comparatively small samples, inadequate statistical power, genetic heterogeneity and the ethnic differences. To comprehensive evaluate the association between *IL10* rs1800872 G>T polymorphism and CAD, we conducted a case-control study of the east Chinese population, along with a meta-analysis on CAD. The results of our case-control study showed that the *IL10* rs1800872 T allele might

be associated with a 0.17-fold decreased risk of CAD in the east Chinese population, and a significantly decreased risk of CAD was also observed in recessive and dominant models. However, the further meta-analysis showed a lack of significant association between *IL10* rs1800872 G>T polymorphism and CAD risk, even upon stratification by ethnicity and control enrolment criteria. The potential factors that may explain the difference included study design and sample population. Our study was not a rigorous case-control study, because the sample was collected from different city, and

thus the controls may not be representative of the underlying source populations. Additionally, the controls in our study were recruited according to their clinical symptoms. Therefore, we cannot exclude the possibility that the apparently healthy elderly controls had underlying CAD, and so confuse and bias the study result. Due to the above limitation, more rigorous researches are needed to elucidate the association.

Heterogeneity is a potential challenge that may affect the explanation of the results. In the present meta-analysis, significant heterogeneity was observed for the association between *IL10* rs1800872 G>T polymorphism and CAD risk among the nine studies. By performing subgroup analysis, significant heterogeneity was still present in all comparisons, except among Caucasian and normal angiography groups in recessive model. The genetic background

might contribute to the observed heterogeneity. Furthermore, the definition of controls was disputable in several available studies. The controls in the studies by Jin et al., Zhou et al. and Koch et al. were recruited on the basis of clear coronary angiographic findings, whereas the other were according to their clinical symptoms. Thus we cannot exclude the possibility that the apparently healthy controls had underlying CAD, and so distort the study results. Therefore, our meta-analysis pinpointed the different selection criteria for controls as a potentially significant source of heterogeneity. As we expect, significant heterogeneity was not detected in normal angiography subgroup in recessive model. Additionally, our sensitivity analysis, excluding two studies deviating from HWE, did not alter the pooled results, indicating that our results were robust and reliable. No apparent publication bias was detected, strengthening this conclusion.

Although this is the first study to describe the *IL10* rs1800872 G>T and the risk for CAD with a relatively large sample size, there are some limitations in this meta-analysis that should be acknowledged when interpreting the results. Firstly, substantial heterogeneity was observed in our meta-analysis. The ethnicity background, lifestyle habits and study designs may contribute to the heterogeneity. Secondly, two studies deviate from Hardy-Weinberg equilibrium expectations. Though, when the analysis was restricted to the studies in HWE, the pooled ORs for the *IL10* rs1800872 G>T and CAD risk remained significant in all genetic models. Thirdly, our outcome was based on unadjusted estimates and a more precise analysis could be performed if individual information (such as gender or age) would have been available.

Our study firstly revealed that the *IL1f7* rs3811047 G>A, *IL28B* rs8099917 T>G and *IL10* rs1800872 G>T variant genotypes were significantly associated with a decreased risk of CAD in an eastern Chinese population, suggesting potential implications for genotyping these SNPs in CAD risk appraisal. The following meta-analysis provide no convincing evidence for the genetic involvement of rs1800872 G>T polymorphism in CAD. Differences in the findings among the present case-control study and meta-analysis call for well-designed with larger sample sizes investigations to further answer the question whether a relationship exists

between rs1800872 G>T polymorphism and CAD risk.

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### Disclosure of conflict of interest

None.

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