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Original Article

Association between three interleukin-10 gene polymorphisms and coronary artery disease risk: a meta-analysis

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Abstract: Background: Previous studies have investigated the associations between interleukin-10 (IL-10) gene polymorphisms (-592C/A, -819C/T and -1082G/A) and risk of coronary artery disease (CAD). However, the results were inconsistent. The aim of this study was to clarify the relationship between *IL-10* polymorphisms and CAD risk by a meta-analysis approach. Methods: The PubMed, Embase, Web of Science, China National Knowledge Infrastructure (CNKI) and Wanfang databases were searched according to predefined criteria for all relevant studies published before June 1, 2015. Pooled odds ratios (ORs) and 95% confidence intervals (95% CIs) were computed to assess the association. Results: 24 eligible studies were enrolled including 9736 CAD patients and 8606 controls. We observed a significant decreased risk of CAD for *IL-10* -819C/T polymorphism (T allele vs. C allele:OR = 0.91, 95% CI = 0.84-0.99; TT vs. CT + CC:OR = 0.82, 95% CI = 0.69-0.98), especially in Asians (T allele vs. C allele:OR = 0.76, 95% CI = 0.60-0.96; TT vs. CC:OR = 0.51, 95% CI = 0.27-0.96; TT vs. CT + CC:OR = 0.62, 95% CI = 0.44-0.88). Moreover, we found *IL-10* -1082G/A polymorphism might contribute to an increased CAD risk in Asians (AA vs. GG:OR = 1.89, 95% CI = 1.36-2.64; AA vs. AG + GG:OR = 1.39, 95% CI = 1.14-1.68) but not in other ethnic groups. However, no significant association between the *IL-10* -592C/A polymorphism and CAD risk was observed. Conclusions: Our results indicated that *IL-10* -819C/T and *IL-10* -1082G/A polymorphisms significantly and race-specifically correlate with CAD risk.

Keywords: Interleukin-10 gene, polymorphisms, coronary artery disease, meta-analysis

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, 2]. The World Health Organization (WHO) estimated that more than 0.7 million deaths in China attributed to CAD annually [3]. CAD is a common complex disease which results from the interactions 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. Moreover, apart from common risk factors, population-based studies have repeatedly reported that genetic susceptibility account for around 50% of the risk for CAD, suggesting that the host genetic background play an important role in the occurrence and development of CAD as well [4, 5]. Therefore, identification of key genetic factors related to CAD risk is important for developing efficient strategies for CAD prediction and therapy.

Inflammation plays a key role in the initiation and development of atherosclerotic vascular disease [6]. Interleukins, a group of cytokines, were recognized as crucial agents involved in the host inflammatory response [7]. Interleukin-10 (IL-10), secreted by Th2 cells as well as by macrophages, is an important anti-inflammatory cytokine with potent deactivating properties on both macrophages and T cells. IL-10 is expressed in human atherosclerotic plaques and function as a protective factor in athero-

sclerosis by balancing pro-inflammatory cytokine activity, decreasing plaque instability and improving prognosis [8, 9]. Previous studies have suggested that decreased IL-10 serum levels are associated with a more unfavorable prognosis in patients with acute coronary syndrome (ACS) [9, 10]. *IL-10* gene is located on chromosome 1, has five exons and has been mapped to the junction between 1q31 and 1q32 [11]. Several polymorphisms in the promoter region of *IL-10* gene, such as *IL-10* -592C/A, *IL-10* -819C/T and *IL-10* -1082G/A polymorphisms, have been reported to be involved in the regulation of *IL-10* gene expression, which might influence the susceptibility to CAD [12]. Numerous studies have been undertaken to investigate the associations between *IL-10* gene polymorphisms and the risk of CAD [11-33], however, no definitive conclusion has yet been reached. To obtain a comprehensive conclusion, we performed a meta-analysis of all of the available case-control studies to systematically evaluate the association of *IL-10* genetic polymorphisms with CAD risk.

Materials and methods

Search strategy and identification of relevant studies

We carried out a comprehensive search of electronic databases including PubMed, Embase, Web of Science, China National Knowledge Infrastructure (CNKI) and Wanfang databases to identify relevant publications reporting on the association between the *IL-10* polymorphisms and CAD risk, with the last search update on June 1, 2015. The following keywords and medical subject headings were employed: ((*IL10* OR Interleukin 10 OR Interleukin-10 OR *IL-10*) AND (coronary artery disease OR CAD OR coronary heart disease OR CHD OR myocardial infarction OR MI OR ischemic heart disease OR IHD OR acute coronary syndrome OR ACS OR angina pectoris OR atherosclerosis)) AND (polymorphism OR variation OR variant OR allele OR mutation). Additional relevant publications were identified by a manual search of bibliographies of retrieved studies and recent reviews.

Studies were included which met the following criteria (with all having to be satisfied): (1) investigation of the association between *IL-10* genetic polymorphisms (-592C/A, -819C/T and -1082G/A) and CAD risk among unrelated subjects; (2) genotypes of the examined polymorphisms were tested in a validated sample size; (3) a case-control study design; (4) providing sufficient information on the genotypes or alleles of the examined polymorphisms to calculate the odds ratio (OR) and its corresponding 95% confidence interval (95% CI); (5) the diagnosis of CAD patients was based on coronary angiography/clinical assessment and controls were free of CAD. Studies were excluded (with one condition being sufficient to do so) if they were meeting abstracts, case reports/series, editorials, review articles, as well as if they published in languages other than English and Chinese. When multiple publications reported the same population, only the most recent one with the largest sample sets was selected for this meta-analysis. Study selection was achieved by two investigators independently, according to the inclusion and exclusion criteria by screening the title, abstract and full-text. Any disagreement was solved by discussion.

Data extraction

Data was extracted from all eligible studies by primary investigators using a standardized extraction form. Extracted forms were reviewed by co-authors and a research assistant to ensure accuracy with dissent settled by consensus. The following information was collected: first author's name, publication year, country and ethnicity of population, outcome, matching status, source of control, number of cases and controls, genotype distributions in cases and controls and the Hardy-Weinberg Equilibrium (HWE) in controls (*P* value). If this were not possible, the authors of the publications were contacted via E-mail for more detailed data.

Quality assessment

The methodological quality of the included studies were accessed by two authors respectively according to the Newcastle Ottawa Scale (NOS) (www.ohri.ca/programs/clinical_epidemiology/oxford.asp). The NOS criteria consist of three aspects: selection, comparability and exposure. Scores ranged from 0 stars (worst) to 9 stars (best) and a score ≥ 7 indicated that a study was of high quality. Dissent was settled as described above.

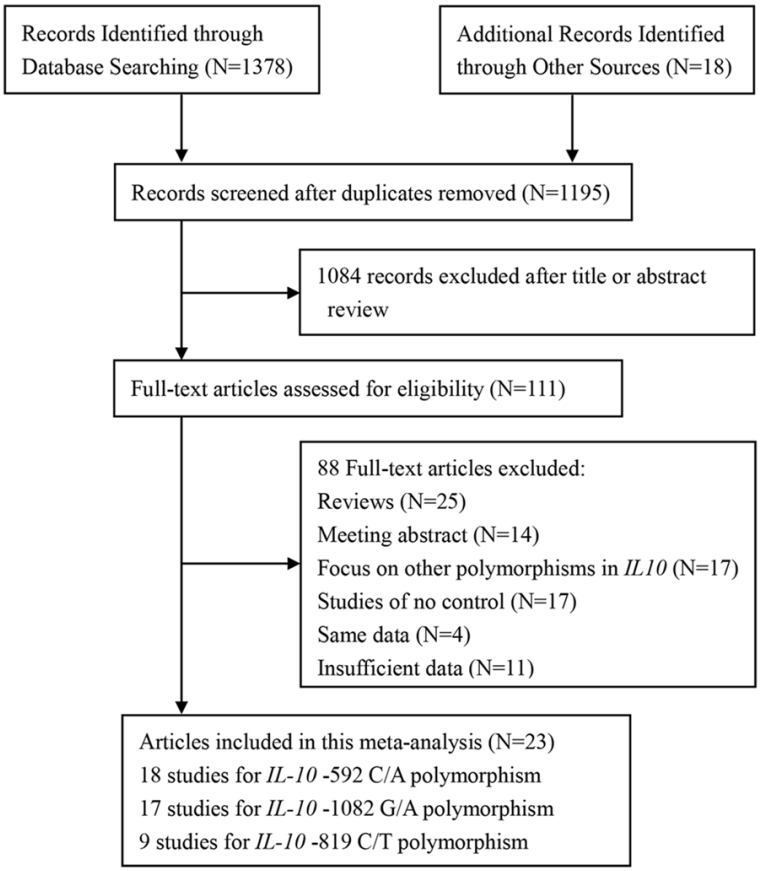

Figure 1. Flow diagram of the search strategy and study selection. The terms "N" in the boxes represent the number of corresponding studies.

Statistical methods

We initially assessed Hardy-Weinberg equilibrium (HWE) among controls subjects by χ^2 test and $P < 0.05$ was considered as significant disequilibrium. The pooled odds ratios (ORs) with their 95% confidence intervals (95% CIs) were calculated to evaluate the strength of the relationship between the *IL-10* gene polymorphisms and CAD risk based on five genetic comparison models: allele model, homozygous model, heterozygous model, dominant model and recessive model. Statistical heterogeneity between eligible studies was evaluated by using the Cochran's Q statistic and I^2 test [34]. $P < 0.1$ and I^2 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. Moreover, a meta-regression was also performed to detect the source of between-study heterogeneity. Predefined subgroup analyses were conducted a priori according to ethnicity (Asian, Caucasian or Mixed), source of control (population-based or hospital-based), study quality (low quality: quality score < 7; high quality: quality score ≥ 7) and matching status (yes or no). A power calculation was performed using Power and Sample Size Calculation version 3.1.2 (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). Sensitivity analyses were performed to look at more narrowly drawn subsets of the studies by removing an individual study or by removing studies with similar feature to assess their influence separately. Begg's funnel plot and Egger's regression test were used to search for publication bias and a P value > 0.05 suggests no significant publication bias have been detected [35]. The fail-safe number (N_{fs}) set at a significance of 0.05 was also calculated to inspect publication bias, according to the formula $N_{fs0.05} = (\sum Z/1.64)^2\text{-}k$, 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. All P values were two sided. All above statistical analyses were performed using STATA software version 12.0 (STATA Corporation, College Station, TX, USA).

Results

Study characteristics

The process of literature retrieval and exclusion was shown in **Figure 1**. The initial comprehensive search identified 1396 potentially relevant articles based on the several predefined criteria, 201 articles were excluded for duplication, and 1084 additional articles were excluded for their unmatched titles or abstracts. After reading the full text of the remaining 111 articles, 88 articles were removed due to reviews, meeting abstract, studies with insufficient data and so on. Since 1 article included two populations, both of them were considered as an indepen-

Table 1. Characteristics of the eligible studies included in this meta-analysis

First author	Year	Country	Ethnicity	Outcome	Matching	Source of control	Sample size		Genotype distribution						P_{HWE}	NOS score
							Case	Control	Case			Control				
IL-10 -592C/A									AA	AC	CC	AA	AC	CC		
Jin [31]	2013	China	Asian	AP, MI	NA	HB	249	132	134	99	16	61	52	19	0.156	5
Fragoso [12]	2011	Mexico	Mixed	MI, UA	Age, gender	PB	389	302	67	179	143	81	139	82	0.167	8
Yu [14]	2012	Korea	Asian	MI, AP	NA	PB	173	313	76	80	17	172	117	24	0.511	6
Nasibullin [15]	2014	Russia	Caucasian	MI	NA	PB	225	257	12	77	136	13	98	146	0.505	6
Zuo [16]	2014	China	Asian	MI, UA	Age, gender	HB	212	218	126	71	15	104	90	24	0.499	7
Babu [17]	2012	India	Asian	ACS	Age, gender	PB	651	432	77	253	321	74	228	130	0.126	7
Karaca [18]	2011	Turkey	Caucasian	CHD	Age	PB	86	88	6	29	51	6	24	58	0.129	7
Ben [19]	2010	Tunisia	Caucasian	CAD	Age, gender	PB	291	291	25	109	156	16	83	188	0.099	8
Afzal [23]	2012	Pakistan	Asian	CAD	Age	PB	93	99	5	84	4	15	81	3	< 0.001	7
Rosner [24]	2005	America	Caucasian	MI	Age, smoking status	PB	522	2089	37	176	309	136	720	1233	0.028	8
Koch [11]	2001	Germany	Caucasian	CAD, MI	Age, gender	HB	1791	340	114	684	993	27	138	175	0.977	7
Donger [25]	2001	France, UK	Caucasian	MI	Age, gender	PB	1107	1082	33	342	612	42	337	576	0.408	8
Lio_a [26]	2004	North Italy	Caucasian	MI	Age	PB	142	153	14	43	85	8	44	101	0.277	7
Lio_b [26]	2004	South Italy	Caucasian	MI	Age	PB	90	110	9	31	50	8	36	66	0.327	7
Biswas [28]	2014	India	Asian	MI	Age, gender	HB	500	500	142	248	110	104	252	144	0.746	7
Cruz [29]	2013	Mexico	Mixed	MI	NA	PB	149	248	28	72	49	41	113	94	0.478	6
Zhou [32]	2012	China	Asian	ACS, SA	NA	HB	118	124	8	40	70	9	33	82	0.039	5
Li [33]	2014	China	Asian	MI	NA	HB	170	153	77	73	20	62	69	22	0.693	5
IL-10 -1082G/A									AA	AG	GG	AA	AG	GG		
Chen [21]	2007	China	Asian	CAD	NA	HB	50	60	39	6	5	35	8	17	< 0.001	5
Fragoso [12]	2011	Mexico	Mxied	MI, UA	Age, gender	PB	389	302	211	142	36	164	113	25	0.380	8
Elsaid [13]	2014	Egypt	Caucasian	IHD	NA	PB	108	143	2	49	22	8	85	5	< 0.001	4
Yu [14]	2012	Korea	Asian	MI, AP	NA	PB	173	313	150	22	1	275	38	0	0.253	6
Babu [17]	2012	India	Asian	ACS	Age, gender	PB	651	432	318	260	73	170	188	74	0.079	7
Karaca [18]	2011	Turkey	Caucasian	CHD	Age	PB	86	88	20	44	22	21	44	23	0.996	7
Ben [19]	2010	Tunisia	Caucasian	CAD	Age, gender	PB	291	291	76	108	101	52	100	76	0.088	8
Lorenzova [20]	2007	Czech Republic	Caucasian	MI	Age	PB	284	568	90	98	40	207	255	106	0.083	7
O'Halloran [22]	2006	Ireland	Caucasian	ACS, SA	NA	PB	1598	386	323	784	491	90	160	136	0.002	6
Afzal [23]	2012	Pakistan	Asian	CAD	Age	PB	93	99	6	77	10	4	92	3	< 0.001	7
Koch [11]	2001	Germany	Caucasian	CAD, MI	Age, gender	HB	1791	340	540	874	377	105	161	74	0.407	7
Donger [25]	2001	France, UK	Caucasian	MI	Age, gender	PB	1107	1082	242	486	256	231	477	244	0.944	8

Lio_a [26]	2004	North Italy	Caucasian	MI	Age	PB	142	153	60	52	30	30	75	48	0.942	7
Lio_b [26]	2004	South Italy	Caucasian	MI	Age	PB	90	110	44	29	17	28	56	26	0.846	7
Ianni [27]	2012	Italy	Caucasian	MI	NA	PB	267	321	68	141	56	78	88	73	< 0.001	6
Cruz [29]	2013	Mexico	Mixed	MI	NA	PB	149	248	55	83	11	125	106	17	0.387	6
Qian [30]	2014	China	Asian	MI, UA	NA	HB	471	197	395	72	4	159	34	4	0.188	5
IL-10 -819C/T									TT	CT	CC	TT	CT	CC		
Fragoso [12]	2011	Mexico	Mixed	MI, UA	Age, gender	PB	389	302	61	175	153	56	146	100	0.833	8
Yu [14]	2012	Korea	Asian	MI, AP	NA	PB	173	313	76	80	17	167	125	21	0.712	6
Ben [19]	2010	Tunisia	Caucasian	CAD	Age, gender	PB	291	291	10	87	191	8	80	162	0.620	8
Afzal [23]	2012	Pakistan	Asian	CAD	Age	PB	93	99	5	84	4	15	81	3	< 0.001	7
Koch [11]	2001	Germany	Caucasian	CAD, MI	Age, gender	HB	1791	340	114	684	993	27	138	175	0.977	7
Donger [25]	2001	France, UK	Caucasian	MI	Age, gender	PB	1107	1082	32	340	611	41	337	576	0.344	8
Lio_a [26]	2004	North Italy	Caucasian	MI	Age	PB	142	153	14	43	85	8	44	101	0.277	7
Lio_b [26]	2004	South Italy	Caucasian	MI	Age	PB	90	110	9	31	50	8	36	66	0.327	7
Cruz [29]	2013	Mexico	Mixed	SMI	NA	PB	149	248	28	69	52	44	119	85	0.833	6

NA, not available; CAD, coronary artery disease; CHD, coronary heart disease; IHD, ischemic heart disease; SMI, silent myocardial ischemia; AP, angina pectoris; MI, myocardial infarction; UA, unstable angina; SA, stable angina; ACS, acute coronary syndrome; HWE, Hardy-Weinberg equilibrium; PB, population-based; HB, hospital-based; NOS, Newcastle Ottawa Scale.

Table 2. Meta-analysis of the association between three *IL-10* polymorphisms and CAD risk

Variables	Case/Control	Allele		Homozygous		Heterozygous		Recessive		Dominant	
		OR (95% CI)	P^{het}	OR (95% CI)	P^{het}	OR (95% CI)	P^{het}	OR (95% CI)	P^{het}	OR (95% CI)	P^{het}
IL-10 -592C/A		A vs. C		AA vs. CC		AC vs. CC		AA vs. AC + CC		AA + AC vs. CC	
Overall	6837/6800	1.03 (0.90-1.19)	< 0.001	1.05 (0.79-1.41)	< 0.001	1.03 (0.87-1.22)	< 0.001	1.00 (0.82-1.23)	< 0.001	1.05 (0.87-1.27)	< 0.001
All in HWE	6104/4488	1.04 (0.88-1.23)	< 0.001	1.09 (0.79-1.52)	< 0.001	1.03 (0.84-1.26)	< 0.001	1.03 (0.83-1.29)	< 0.001	1.06 (0.85-1.32)	< 0.001
Ethnicity											
Caucasian	2392/4279	1.05 (0.92-1.21)	0.024	1.03 (0.84-1.27)	0.190	1.00 (0.90-1.11)	0.187	1.03 (0.84-1.26)	0.380	1.05 (0.90-1.22)	0.058
Asian	2166/1971	1.04 (0.78-1.39)	< 0.001	1.07 (0.60-1.89)	< 0.001	1.09 (0.69-1.72)	< 0.001	0.99 (0.71-1.39)	< 0.001	1.12 (0.68-1.84)	< 0.001
Mixed	538/550	0.88 (0.52-1.49)	0.003	0.77 (0.29-2.09)	0.006	0.93 (0.57-1.52)	0.085	0.79 (0.39-1.61)	0.028	0.88 (0.46-1.69)	0.016
Source of control											
PB	3624/5020	0.97 (0.81-1.17)	< 0.001	0.92 (0.64-1.32)	< 0.001	0.95 (0.76-1.20)	< 0.001	0.91 (0.70-1.18)	0.014	0.96 (0.75-1.22)	< 0.001
HB	3213/1780	1.13 (0.92-1.40)	< 0.001	1.29 (0.86-1.93)	0.005	1.10 (0.94-1.29)	0.164	1.12 (0.84-1.49)	0.004	1.24 (0.93-1.66)	0.014
Matched											
Yes	5753/5573	1.02 (0.85-1.22)	< 0.001	0.99 (0.68-1.44)	< 0.001	0.98 (0.79-1.21)	< 0.001	0.99 (0.75-1.30)	< 0.001	1.00 (0.80-1.27)	< 0.001
No	1084/1227	1.07 (0.87-1.31)	0.031	1.21 (0.90-1.63)	0.134	1.13 (0.91-1.41)	0.254	1.00 (0.82-1.23)	0.131	1.13 (0.92-1.39)	0.112
Quality score											
High	5753/5573	1.02 (0.85-1.22)	< 0.001	0.99 (0.68-1.44)	< 0.001	0.98 (0.79-1.21)	< 0.001	0.99 (0.75-1.30)	< 0.001	1.00 (0.80-1.27)	< 0.001
Low	1084/1227	1.07 (0.87-1.31)	0.031	1.21 (0.90-1.63)	0.134	1.13 (0.91-1.41)	0.254	1.00 (0.82-1.23)	0.131	1.13 (0.92-1.39)	0.112
IL-10 -1082G/A		A vs. G		AA vs. GG		AG vs. GG		AA vs. AG + GG		AA + AG vs. GG	
Overall	7518/4813	1.09 (0.97-1.23)	< 0.001	1.20 (0.95-1.51)	< 0.001	1.04 (0.84-1.28)	< 0.001	1.13 (0.94-1.36)	< 0.001	1.11 (0.91-1.35)	< 0.001
All in HWE	5439/3931	1.12 (0.98-1.28)	< 0.001	1.27 (0.99-1.62)	0.005	1.02 (0.90-1.16)	0.779	1.18 (0.97-1.45)	< 0.001	1.09 (0.97-1.22)	0.257
Ethnicity											
Caucasian	5542/3162	1.08 (0.94-1.24)	< 0.001	1.16 (0.90-1.50)	0.002	1.01 (0.79-1.30)	< 0.001	1.14 (0.89-1.45)	< 0.001	1.08 (0.87-1.33)	0.001
Asian	1438/1101	1.27 (0.94-1.71)	0.020	1.89 (1.36-2.64)	0.188	1.07 (0.47-2.44)	0.069	1.39 (1.14-1.68)	0.307	1.29 (0.54-3.06)	0.022
Mixed	538/550	0.85 (0.64-1.14)	0.133	0.82 (0.52-1.30)	0.588	0.97 (0.61-1.54)	0.517	0.77 (0.45-1.32)	0.036	0.90 (0.58-1.39)	0.930
Source of control											
PB	5033/3903	1.07 (0.93-1.22)	< 0.001	1.16 (0.90-1.50)	< 0.001	1.00 (0.79-1.27)	< 0.001	1.12 (0.89-1.41)	< 0.001	1.06 (0.85-1.32)	< 0.001
HB	2485/910	1.24 (0.86-1.80)	0.014	1.58 (0.64-3.90)	0.055	1.11 (0.84-1.48)	0.362	1.07 (0.88-1.30)	0.140	1.56 (0.67-3.63)	0.072
Matched											
Yes	4739/3272	1.15 (1.00-1.32)	< 0.001	1.28 (0.99-1.65)	0.005	1.00 (0.88-1.13)	0.409	1.30 (1.05-1.61)	< 0.001	1.09 (0.92-1.30)	0.079
No	2779/1541	0.99 (0.77-1.28)	0.001	0.98 (0.56-1.74)	0.004	1.10 (0.59-2.03)	< 0.001	0.87 (0.65-1.17)	0.019	1.06 (0.60-1.90)	<0.001
Quality score											
High	4739/3272	1.15 (1.00-1.32)	< 0.001	1.28 (0.99-1.65)	0.005	1.00 (0.88-1.13)	0.409	1.30 (1.05-1.61)	< 0.001	1.09 (0.92-1.30)	0.079
Low	2779/1541	0.99 (0.77-1.28)	0.001	0.98 (0.56-1.74)	0.004	1.10 (0.59-2.03)	< 0.001	0.87 (0.65-1.17)	0.019	1.06 (0.60-1.90)	< 0.001
IL-10 -819C/T		T vs. C		TT vs. CC		CT vs. CC		TT vs. CT+CC		TT+CT vs. CC	
Overall	4098/2769	0.91 (0.84-0.99)	0.288	0.82 (0.67-1.01)	0.283	0.92 (0.82-1.03)	0.952	0.82 (0.69-0.98)	0.241	0.91 (0.81-1.01)	0.671
All in HWE	4005/2670	0.91 (0.84-0.99)	0.232	0.83 (0.68-1.03)	0.325	0.92 (0.82-1.03)	0.915	0.85 (0.71-1.02)	0.415	0.91 (0.81-1.02)	0.579
Ethnicity											
Caucasian	3294/1807	0.95 (0.85-1.05)	0.236	0.90 (0.68-1.19)	0.229	0.94 (0.83-1.08)	0.843	0.92 (0.69-1.21)	0.304	0.94 (0.83-1.06)	0.518

Asian	266/412	0.76 (0.60-0.96)	0.755	0.51 (0.27-0.96)	0.412	0.79 (0.42-1.49)	0.985	0.62 (0.44-0.88)	0.179	0.67 (0.36-1.23)	0.951
Mixed	538/550	0.89 (0.75-1.06)	0.286	0.82 (0.57-1.16)	0.311	0.84 (0.64-1.10)	0508	0.90 (0.66-1.24)	0.418	0.83 (0.65-1.07)	0.371
Source of control											
PB	2134/2116	0.94 (0.85-1.04)	0.322	0.88 (0.69-1.13)	0.227	0.94 (0.82-1.07)	0.894	0.89 (0.71-1.12)	0.181	0.94 (0.82-1.06)	0.621
HB	1964/653	0.83 (0.71-0.97)	0.358	0.68 (0.47-1.00)	0.506	0.86 (0.69-1.09)	0.792	0.73 (0.55-0.96)	0.632	0.83 (0.67-1.03)	0.479
Matched											
Yes	3776/2208	0.92 (0.84-1.00)	0.304	0.82 (0.65-1.04)	0.239	0.92 (0.82-1.04)	0.869	0.84 (0.67-1.04)	0.209	0.91 (0.81-1.02)	0.569
No	322/561	0.86 (0.64-1.17)	0.137	0.81 (0.52-1.27)	0.184	0.90 (0.61-1.32)	0.670	0.80 (0.59-1.08)	0.172	0.87 (0.61-1.25)	0.338
Quality score											
High	3776/2208	0.92 (0.84-1.00)	0.304	0.82 (0.65-1.04)	0.239	0.92 (0.82-1.04)	0.869	0.84 (0.67-1.04)	0.209	0.91 (0.81-1.02)	0.569
Low	322/561	0.86 (0.64-1.17)	0.137	0.81 (0.52-1.27)	0.184	0.90 (0.61-1.32)	0.670	0.80 (0.59-1.08)	0.172	0.87 (0.61-1.25)	0.338

P^{het}, *P* value for heterogeneity.

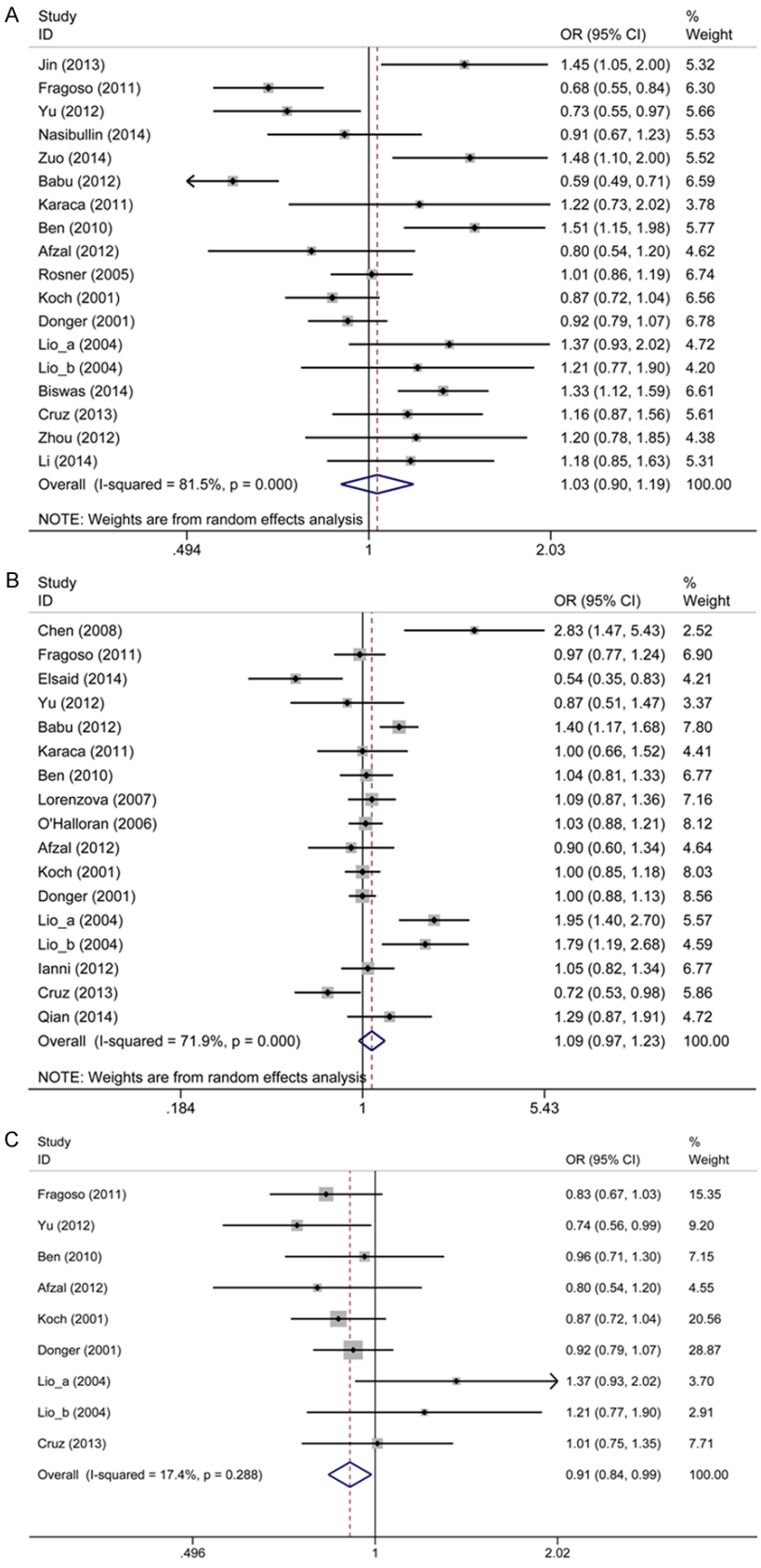

Figure 2. Forest plot of the risk of CAD associated with the *IL-10* gene polymorphisms under allele model. A. *IL-10* -592C/A polymorphism. B. *IL-10* -1082G/A polymorphism. C. *IL-10* -819-C/T polymorphism. The solid diamonds and horizontal lines correspond to the study-specific ORs and 95% CIs. The gray areas reflect the study-specific weight. The hollow diamonds represent the pooled ORs and 95% CIs of the overall population. The vertical solid lines show the OR of 1 and the vertical dashed lines indicate the corresponding pooled OR.

dent study [26]. Finally, 24 studies were included in our meta-analysis, in total of 9736 CAD patients and 8606 controls [11-33]. The general characteristics of all eligible studies were summarized in **Table 1**. There were 10 studies based on Asian population [14, 16, 17, 21, 23, 28, 30-33], 12 studies conducted in Caucasian population [11, 13, 15, 18-20, 22, 24-27] and 2 studies from mixed population [12, 29]. For -592C/A polymorphism of *IL-10*, we included 18 studies with a total of 6837 cases and 6800 controls [11, 12, 14-19, 23-26, 28, 29, 31-33]. 17 studies about -1082G/A polymorphism consisting of 7518 cases and 4813 controls [11-14, 17-23, 25-27, 29, 30], and 9 studies of -819C/T polymorphism involving 4098 cases and 2769 controls were included as well [11, 12, 14, 19, 23, 25, 26, 29]. These 3 polymorphisms were found to occur in frequencies consistent with HWE in the control populations of the vast majority of the published studies.

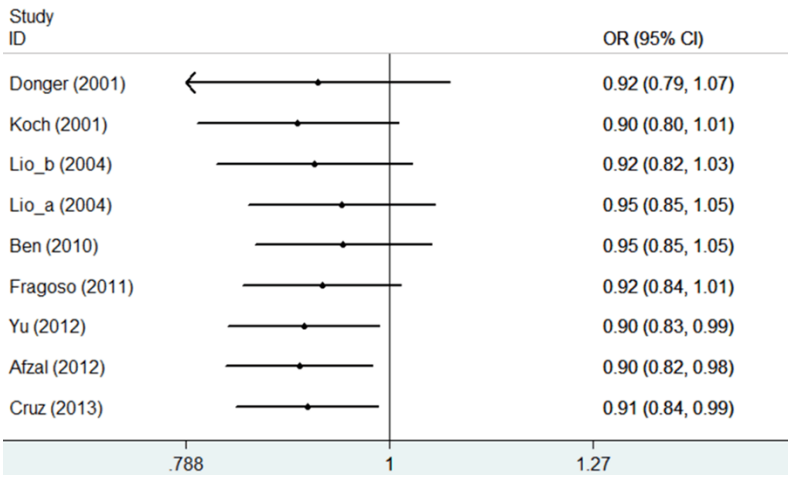

Figure 3. Cumulative meta-analysis of the *IL-10* -819C/T polymorphism and CAD. The points and horizontal lines represent the point estimates and the 95% CIs of the pooled results after the inclusion of each additional study in the calculations. The vertical solid lines show the OR of 1. The studies have been sorted in order of year of publications. The CIs typically narrow with the addition of more studies over the years, suggesting that substantial heterogeneity does not exist.

Overall analyses

Table 2 and **Figure 2** showed the main meta-analysis results of relationships between three *IL-10* polymorphisms and CAD risk. A random-effects model was applied to all the genetic models in the study of *IL-10* -592C/A and -1082G/A polymorphisms, while a fixed-effect model was chosen for all comparison models in *IL-10* -819C/T polymorphism according to the *P* values for heterogeneity. The pooled estimates failed to identify any significant association of CAD with the *IL-10* -592C/A (allele: OR = 1.03, 95% CI = 0.90-1.19; homozygous: OR = 1.05, 95% CI = 0.79-1.41; heterozygous: OR = 1.03, 95% CI = 0.87-1.22; recessive: OR = 1.00, 95% CI = 0.82-1.23 and dominant comparing: OR = 1.05, 95% CI = 0.87-1.27) or *IL-10* -1082G/A polymorphisms (allele: OR = 1.09, 95% CI = 0.97-1.23; homozygous: OR = 1.20, 95% CI = 0.95-1.51; heterozygous: OR =1.04, 95% CI = 0.84-1.28; recessive: OR = 1.13, 95% CI = 0.94-1.36 and dominant comparing: OR = 1.11, 95% CI = 0.91-1.35). However, a significant relationship between *IL-10* -819C/T polymorphism and CAD risk was observed in allelic comparison and recessive model (allele: OR = 0.91, 95% CI = 0.84-0.99; recessive: OR = 0.82, 95% CI = 0.69-0.98). If we set $\alpha = 0.05$, based on the data set for -819 T allele, we have a 72% power to detect an OR of 0.91. We also performed a cumulative meta-analysis based on publication date, which further confirmed our results (**Figure 3**). Sensitivity analyses were performed by excluding studies with controls deviate from HWE. The results show that the associations between the *IL-10* -592C/A, *IL-10* -1082G/A and *IL-10* -819C/T polymorphisms and CAD risk were not significantly altered, suggesting that the results were robust and reliable.

Subgroup analyses

To exclude the effect of potential confounding factors, subgroup analyses were introduced to further elucidate the relationship between *IL-10* polymorphisms and the risk of CAD. We divided included studies into 2 to 3 subgroups according to ethnicity, source of control, matching status and quality score (**Table 2**). For the *IL-10* -592C/A polymorphism, no significant association was still found in any subgroup analyses. For the *IL-10* -1082G/A polymorphism, the subgroup analysis by ethnicity revealed a significantly increased CAD risk for Asians (homozygous: OR = 1.89, 95% CI = 1.36-2.64, and recessive comparing: OR = 1.39, 95% CI = 1.14-1.68). In the subgroup analysis of the quality score, significant association was also found in high-quality studies (allele: OR = 1.15, 95% CI = 1.00-1.32, and recessive comparing: OR = 1.30, 95% CI = 1.05-1.61). Similar results were observed in the subgroup analysis by matching status. No significant association was found in the subgroup analysis by source of control. For the *IL-10* -819C/T polymorphism, we observed a significantly decreased risk of CAD for Asians (allele: OR = 0.76, 95% CI = 0.60-0.96; homozygous: OR = 0.51, 95% CI = 0.27-0.96, and recessive comparing: OR = 0.62, 95% CI = 0.44-0.88) but no other ethnic groups, when the analysis was stratified by ethnicity. Subgroup analysis by source of control showed that significant association was also found in the HB group (allele: OR = 0.83, 95% CI = 0.71-0.97; homozygous: OR = 0.68, 95% CI = 0.47-1.00, and recessive comparing: OR = 0.73, 95% CI = 0.55-0.96). No significant association was ob-

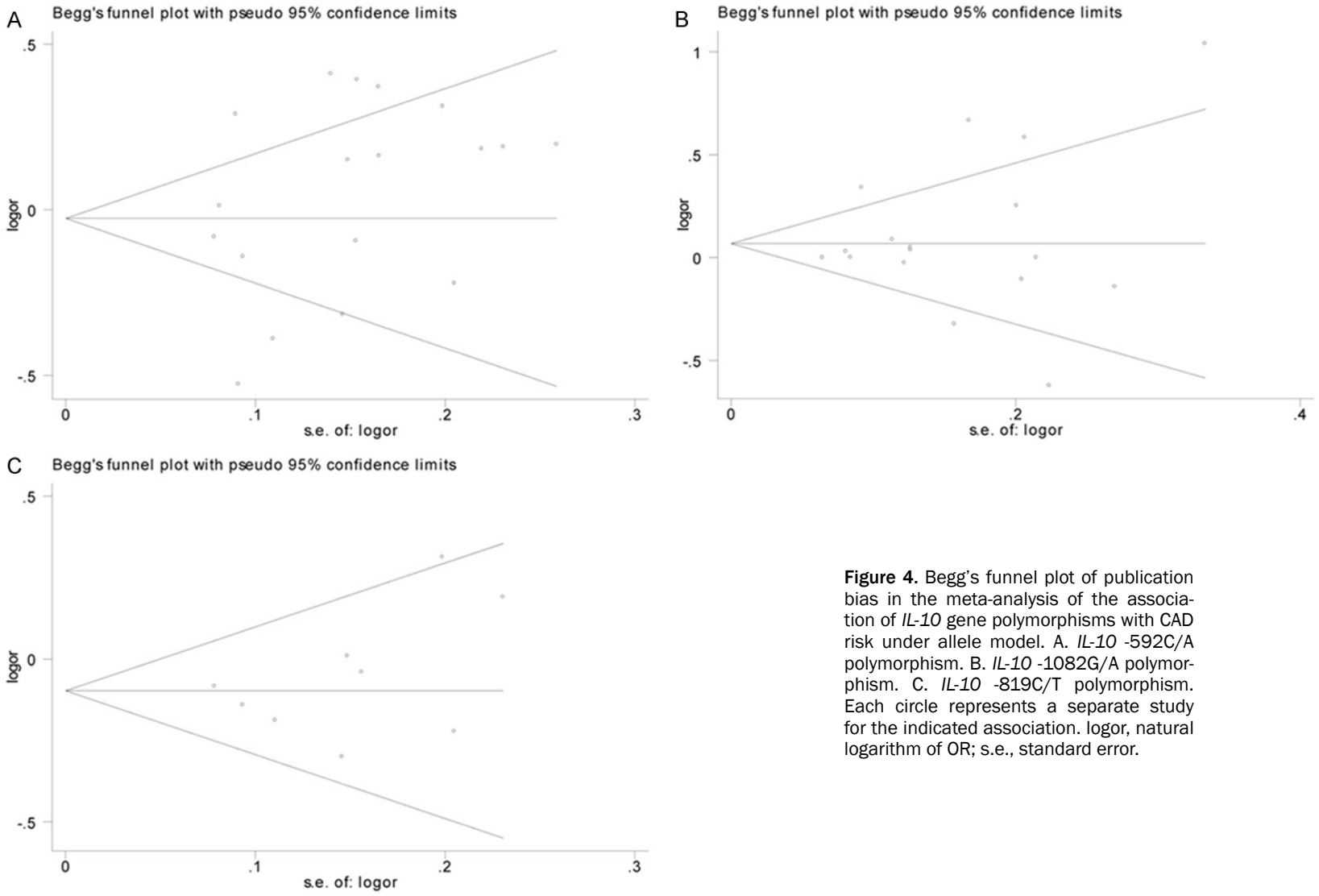

Figure 4. Begg's funnel plot of publication bias in the meta-analysis of the association of *IL-10* gene polymorphisms with CAD risk under allele model. A. *IL-10* -592C/A polymorphism. B. *IL-10* -1082G/A polymorphism. C. *IL-10* -819C/T polymorphism. Each circle represents a separate study for the indicated association. logor, natural logarithm of OR; s.e., standard error.

served in the subgroup analyses by matching status or quality score.

Sources of heterogeneity

Heterogeneity between studies in each comparison model was shown in **Table 2**. No significant heterogeneity was found for *IL-10* -819C/T polymorphism, but for *IL-10* -592C/A or *IL-10* -1082G/A polymorphisms, substantial heterogeneities were detected in all genetic models (all comparisons $P < 0.001$). With regard to *IL-10* -1082G/A polymorphism and CAD, meta-regression revealed that ethnicity might be the sources of between-study heterogeneity under homozygous genetic model ($t = 1.80$, $P = 0.095$), which was consistent with subgroup analyses result in homozygous genetic model. As for *IL-10* -592C/A polymorphism, meta-regression showed that ethnicity, source of controls, matching status and quality score did not contributed to the source of heterogeneity.

Publication bias

Begg's funnel plot and Egger's test were performed to evaluate the potential publication bias of literatures. The shapes of the funnel plots show no evidence of obvious asymmetry (**Figure 4**). The Egger's test results did not support the existence of publication bias. The $N_{fs0.05}$ values for *IL-10* -592C/A, *IL-10* -1082G/A and *IL-10* -819C/T polymorphisms were 322, 128 and 24, respectively, which were consistently greater than the number of studies included in this meta-analysis.

Discussion

The core pathological mechanism of CAD is atherosclerosis and IL-10 play a key role in atherosclerotic plaque formation and progression. Previous studies have shown that the decreased serum IL-10 levels are associated with a poor prognosis in patients with ACS [1, 2]. Three variants within the promoter region of *IL-10* gene (*IL-10* -819C/T, *IL-10* -592C/A and *IL-10* -1082G/A) could influence the production of IL-10, which may be associated with increased risk of CAD. Although a number of studies investigated this issue, the conclusions were inconsistent. Thus, we conducted a meta-analysis to obtain a more precise result.

Although data from some individual studies suggested a relationship [12, 14, 16, 17, 19, 23, 28, 31], the overall result of the present meta-analysis argued against an association of *IL-10* -592C/A polymorphism with CAD risk in all genetic models. Subgroup analyses also did not detect a significant association between *IL-10* -592C/A and CAD risk in any of the comparisons. This meta-analysis had sufficient statistical power to detect such genetic effect. Thus, it is reasonable to surmise that *IL-10* -592C/A polymorphism itself exhibits null contribution to the susceptibility of CAD, or its influence on CAD is limited and depends on neighboring variants that compensate or dilute the variation under study.

Likewise, the overall pooled result show a lack of relationship between *IL-10* -1082G/A polymorphism and CAD risk. However, a subgroup analysis by ethnicity revealed that *IL-10* -1082G/A polymorphism was associated with increased risk of CAD in Asian population under homozygous and recessive models, while no effect was observed in other ethnic groups. This result suggests that association between *IL-10* -1082G/A polymorphism and increased CAD risk may be ethnicity and model dependent. In addition, it is worth noting that our results were in accord with the results of the previous study by Chao [36] and inconsistent with the study by Wang [37]. There were two main differences between the prior study and ours. First, apart from ethnicity, the influence of factors such as study quality, source of control and matching status were not stated to investigate the potential associations in the subtype analysis. Second, the literature searches of the Wang's meta-analysis were performed before February 2010. Since then, several additional studies of the *IL-10* -1082G/A polymorphism and risk of CAD were published. Thus, the sample was larger and the results of our meta-analysis were more reliable than those of previous studies.

As for *IL-10* -819C/T polymorphism, our result showed that the individuals who carry the TT genotype have 18% decreased risk of CAD compared with the C allele carriers (CT+CC), and a significantly decreased risk of CAD was also found in allele model. This may be due to the fact that *IL-10* -819 T allele potently alter the *IL-10* gene activity resulting in a marked increase of plasma IL-10 concentration. In addition, subgroup analysis by ethnicity showed that the association between *IL-10* -819C/T polymorphism and CAD risk was significant in

Asians, but not in other ethnic groups. However, in our meta-analysis for *IL-10* -819C/T polymorphism, only two study were conducted in Asian race totally. So, this finding should be interpreted with caution.

In order to make the conclusion more credible, we performed the publication bias analysis and sensitivity analysis according to Cochrane protocol. Funnel plots suggested that no obvious publication bias was observed. The $N_{fs0.05}$ for all polymorphisms were greater than the number of studies included in this meta-analysis, also indicating a low probability of publication bias. The sensitivity analysis by excluding studies with controls deviate from HWE showed that the results are robust and convincing. Meanwhile, the existence of heterogeneity among the available studies affects the reliability of the results in a large extent. Thus, we performed a subgroup analyses according to ethnicity, source of controls, quality score, and matching status. As for *IL-10* -1082G/A polymorphism, results from subgroup analysis suggested that the ethnicity might be a source of heterogeneity, which were confirmed by meta-regression. As for *IL-10* -592C/A polymorphism, the heterogeneity between the studies could not be explained. Some other factors, such as different selective criteria of controls and study sample size, may have a certain influence.

Although this is the first meta-analysis to our knowledge to investigate the association between *IL-10* -819C/T and *IL-10* -592C/A polymorphisms and CAD with a relatively large sample size, there are some limitations in this meta-analysis that should be acknowledged when interpreting the results. First, we only included the studies written in English and Chinese, and might miss some investigations written in other languages. Second, substantial heterogeneity was observed in the analysis of *IL-10* -592C/A and *IL-10* -1082G/A polymorphisms with CAD risk. The lifestyle habits, geographical location and study designs may contribute to the heterogeneity. Third, several studies deviate from Hardy-Weinberg equilibrium expectations. Though, when the analysis was restricted to the studies in HWE, the pooled results did not alter significantly. Fourth, our outcome was based on unadjusted estimates and a more precise analysis could be performed if individual information (such as age, family history and environment factors) would have been available.

In summary, this meta-analysis indicated that *IL-10* -819C/T polymorphism might be a protective factor for CAD, particularly in Asians, whereas *IL-10*-1082G/A polymorphism might be associated with increased CAD risk among Asians. In addition, *IL-10* -592C/A polymorphism was not likely to exert any influence on the susceptibility of CAD. Considering the limitations listed above, future well-designed studies with large sample size should be conducted to validate our findings. Moreover, other interleukin polymorphisms and gene-gene interactions should also be considered in future studies.

Disclosure of conflict of interest

None.

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