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SYSTEMATIC REVIEW AND META-ANALYSIS

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Association Between 3 IL-10 Gene Polymorphisms and Cardiovascular Disease Risk

Systematic Review With Meta-Analysis and Trial Sequential Analysis

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Abstract: Previous studies have yielded controversial results related to the contribution of interleukin 10 (*IL-10*) gene polymorphisms (*IL-10* -592C/A, *IL-10* -1082G/A, and *IL-10* -819C/T) in the progression of cardiovascular disease (CVD). Thus, we performed a meta-analysis to summarize this situation.

Eligible studies were retrieved by searching PubMed, Embase, Web of Science, and Cochrane Library with the last search up to July 7, 2015. Data were pooled by odds ratios (ORs) and their 95% confidence intervals (CIs). False-positive report probability (FPRP) analysis was conducted for all significant findings. Genotype-based mRNA expression analysis was also performed using data from 270 individuals with different ethnicities.

Finally, 19 studies for *IL-10* -592C/A polymorphism (7284 cases and 7469 controls), 21 studies for *IL-10* -1082G/A polymorphism (8263 cases and 5765 controls), and 12 studies for *IL-10* -819C/T polymorphism (4502 cases and 3190 controls) were included in the meta-analyses. With respect to *IL-10* -819C/T polymorphism, statistically significant decreased CVD risk was found when all studies were pooled into the meta-analysis (T vs C: OR = 0.91, 95% CI = 0.84–0.98; TT + TC vs CC: OR = 0.90, 95% CI = 0.81–1.00). Subgroup analyses stratified by disease subtype suggested the -819C/T polymorphism was significantly associated with a decreased CAD risk (T vs C: OR = 0.90, 95% CI = 0.83–0.97; TT vs CC: OR = 0.81, 95% CI = 0.66–1.00; TT vs TC + CC: OR = 0.82, 95% CI = 0.69–0.98; TT + TC vs CC: OR = 0.89, 95% CI = 0.80–0.99), which was noteworthy finding as evaluated by FPRP. However, with regard to *IL-10* -592C/A and *IL-10* -1082G/A polymorphisms, no significant association with CVD risk was observed in the overall and subgroup analyses.

In conventional meta-analyses, the results suggested that *IL-10* -819C/T polymorphism was associated with decreased risk of CVD, especially CAD outcome, whereas *IL-10* -592C/A and *IL-10* -1082G/A polymorphisms might have no influence on the susceptibility of CVD. However, trial sequential analysis does not allow us to draw any solid conclusion for the association between *IL-10* -592C/A or *IL-10* -1082G/A polymorphism and CVD risk. Further large and well-designed studies are still needed.

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Abbreviations: CAD = coronary artery disease, CI = confidential interval, CVD = cardiovascular disease, FPRP = False-positive report probability, HWE = Hardy-Weinberg equilibrium, IL-10 = interleukin 10, IS = ischemic stroke, OR = odds ratio, PCR = polymerase chain reaction, SNP = single-nucleotide polymorphism, TSA = trial sequential analysis.

INTRODUCTION

Cardiovascular diseases (CVDs) such as coronary artery disease (CAD) and stroke are the leading cause of death worldwide and represent a public health challenge in both industrialized and developing countries.[1,2] A number of clinical risk factors for CVD have been identified for decades, involving obesity, dyslipidemia, hypertension, diabetes, and a sedentary lifestyle. Nevertheless, the molecular basis of CVD is complex and linked to a broad range of biological pathways, including lipid and glucose metabolism, vascular repair, and angiogenesis.[3] Apart from these, more and more evidence showed that inflammatory molecules might take part in the pathogenesis of CVD as well.[4,5]

Inflammation has been shown to involve in the manifestation and development of arterial thrombotic diseases.[6,7] Interleukins, a group of cytokines, were recognized as crucial agents involved in the host inflammatory response.[8] 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.[9] IL-10 exerts a negative modulator effect on the inflammatory response by inhibiting cytokine synthesis.[5,10] Because of its anti-inflammatory function, IL-10 is thought to be involved in arterial thrombotic diseases and further illustrated by epidemiologic studies, which recognized an association between lower levels of plasma IL-10 and increased risk of several end points of CVD such as acute coronary disease (ACS) and ischemic stroke (IS).[5,11,12] Previous studies have reported that approximately 75% of individual difference in IL-10 secretion is determined by genetic factors and controlled at transcriptional level.[13] *IL-10* gene is located on chromosome 1, has 5 exons, and has been mapped to the junction between 1q31 and 1q32.[14] Three single-nucleotide polymorphisms (SNPs) (G-1082A, C-819T, and C-592A) in the promoter region of *IL-10* were found to be associated with transcription activity of *IL-10* gene and levels of plasma IL-10.[13,15] Owing to their important roles, they were extensively studied and anticipated to be involved in arterial thrombotic diseases. This is supported by several studies that

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observe an increased risk of CVD in *IL-10* -1082 A allele carriers.[4,16,17] However, such associations could not be confirmed in other studies.[10,14,18,19] The associations between *IL-10* -592C/A and *IL-10* -819C/T polymorphisms and CVD were not conclusive as well.[10,13–17,19–32] Therefore, we performed this systematic review with meta-analysis and trial sequential analysis (TSA) of all the published case–control studies in the hope of providing more precise evidence.

METHOD

Search Strategy and Identification of Relevant Studies

We carried out a comprehensive search of electronic databases including PubMed, Embase, Web of Science, and Cochrane Library to identify relevant publications reporting on the association between the *IL-10* polymorphisms and CVD risk, with the last search update on July 7, 2015. The following keywords and medical subject headings were employed: (''interleukin 10'' or ''interleukin-10'' or ''IL-10'' or ''IL 10''), (''acute coronary syndrome'' or ''myocardial infarction'' or ''coronary artery disease'' or ''coronary heart disease'' or ''ischemic heart disease'' or ''cardiovascular disease'' or ''cardiovascular'' or ''stroke'' or ''myocardial ischaemia'' or ''myocardial ischemia'' or ''cerebral ischemia'' or ''cerebral ischaemia'' or ''cerebral infarction'' or ''brain infarction''), and (''polymorphism'' or ''variation'' or ''variant'' or ''allele'' or ''mutation'' or ''SNP''). Additional relevant publications were identified by a manual search of bibliographies of retrieved studies and recent reviews. Ethical approval and informed consent were not necessary because our analyses were based on data from previously published studies.

Studies were included that met the following criteria: investigation of the association between *IL-10* -592C/A, *IL-10* -1082G/A, or *IL-10* -819C/T polymorphism and CVD among unrelated subjects; case–control design; sufficient information provided to calculate odds ratio (OR) and the corresponding confidence interval (CI). In addition, exclusion criteria were as follows: meeting abstracts, case reports, reviews, or editorials; overlapping data; studies were published in languages other than English and Chinese. The articles with the largest dataset were chosen when there were multiple publications from the same population. Two investigators selected the studies according to the above criteria, and disagreements were resolved by consensus.

Data Extraction

Data were 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, genotyping methods, number of cases and controls, genotype distributions in cases and controls, and the Hardy-Weinberg Equilibrium (HWE) in controls (*P*). If these were not possible, the authors of the publications were contacted via E-mail for more detailed data.

Genotype-based mRNA Expression Analysis

The genotypes data for *IL-10* -592C/A, *IL-10* -1082G/A, and *IL-10* -819C/T polymorphisms were available from the HapMap (http://hapmap.ncbi.nlm.nih.gov/) for 270 subjects with 3 different ethnicities and their corresponding mRNA expression levels data were available from SNPexp (http://app3.titan.uio.no/biotools/tool.php?app=snpexp) as described previously.[33,34]

Quality Assessment

The methodological quality of the included studies was accessed by 2 authors respectively according to the Newcastle Ottawa Scale (NOS) (www.ohri.ca/programs/clinical_epidemiology/oxford.asp).[35] The NOS criteria consist of 3 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.

Statistical Analyses

We initially assessed HWE among control subjects by χ^2 test and $P < 0.05$ was considered as significant disequilibrium. The pooled ORs with their 95% CIs were calculated to evaluate the strength of the association between the *IL-10* gene polymorphisms and CVD risk based on 5 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.[36] $P < 0.1$ 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. Predefined subgroup analyses were conducted a priori according to ethnicity (Asian, white, or mixed), disease subtype (CAD or stroke), and quality score (low quality: score <7; high quality: score ≥ 7). 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 funnel plot and Egger regression test were used to search for publication bias and a P value >0.05 suggests no significant publication bias has been detected.[37] The fail-safe number (N_{fs}) set at a significance of 0.05 was also calculated to inspect publication bias, according to the formula $N_{fs0.05} = (\sum Z/1.64)^2$-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 each statistically significant association identified, we estimated the false-positive reporting probability (FPRP).[38] The FPRP value is determined by the P value, the prior probability for the association, and statistical power. We set 0.2 as an FPRP threshold and assigned a prior probability of 0.1 to detect an OR of 1.50/0.67 for alleles with a risk/protective effect. Only the results with FPRP values <0.2 were referred as noteworthy.

All P values were 2-sided. All above statistical analyses were performed using STATA software version 12.0 (STATA Corporation, College Station, TX).

TSA

Meta-analyses may result in type I errors owing to an increased risk of random error when sparse data are analyzed and to repeated significance testing when a cumulative meta-analysis is updated with new trials. TSA has been introduced to control the risk of type I error by estimating the required information size and an adjusted threshold for statistical

significance. [39–41] A required information size was calculated with the adjustment by diversity (D^2) between trials. We performed TSA with a desire to maintain an overall 5% risk of a type I error and 20% of the type II error (a power of 80%).[42] As for *IL-10* -592C/A, *IL-10* -1082G/A, and *IL-10* -819C/T polymorphisms, the required information size was calculated based on a relative risk increase of 6%, a relative risk reduction of 2%, a relative risk reduction of 10%, respectively, with low-risk bias (taking the data of dominant model for example). For *IL-10* -819C/T polymorphism and CAD, we observed an 11% relative risk reduction. The control event proportion was calculated from the actual meta-analyses.

When the cumulative Z-curve crosses the trial sequential monitoring boundary, a sufficient level of evidence may have been reached and further trials are unnecessary. If the Z curve does not cross any of the boundaries and the required information size has not been reached, evidence to reach a conclusion is insufficient.[43] We used software Trial Sequential Analysis (version 0.9, http://www.ctu.dk/tsa/) and provided the 95% CIs adjusted for sparse data or repetitive testing, which we describe as the TSA-adjusted 95% CIs.

RESULTS

The Main Characteristics of Included Studies

The process of literature retrieval and exclusion was shown in Figure 1. The initial comprehensive search identified a total of 1087 potentially relevant articles, 227 articles were excluded for duplication, and 789 additional articles were excluded for their unmatched titles or abstracts. After reading the full text of the remaining 71 articles, 44 articles were removed due to reviews, meeting abstract, studies with insufficient data, and so on. Since 1 article included 2 populations, both of them were considered as an independent study.[27] Finally, 27 articles including 28 case–control studies, involving a total sample size of 20875, were included in our meta-analysis.[4,10,13–32,44–48] Detailed characteristics and genotype distributions of included studies were summarized in Tables 1 and 2, respectively.[4,10,13–32,44–48] The 28 studies concerned *IL-10* -592C/A polymorphism, *IL-10* -819C/T polymorphism, *IL-10* -1082G/A polymorphism, respectively. These 3 polymorphisms were found to occur in frequencies consistent with HWE in the control populations of the vast majority of the published studies. There were 10 studies based on Asian population,[4,13,16,17,20,23,25,28,32,48] 16 studies conducted in white population,[10,14,18,19,21,22,26,27,29–31,44–47] and 2 studies from mixed population.[15,24] Among these included studies, cases were generally recruited in referral centers with documented CAD or stroke, and the controls were without any direct evidence of overt disease. The number of cases among all selected studies varied from 86 to 1791, whereas the numbers of controls varied from 48 to 2089. All the studies included met quality criteria ranging from 4 to 9 (Supplemental Table 1, http://links.lww.com/MD/A696).

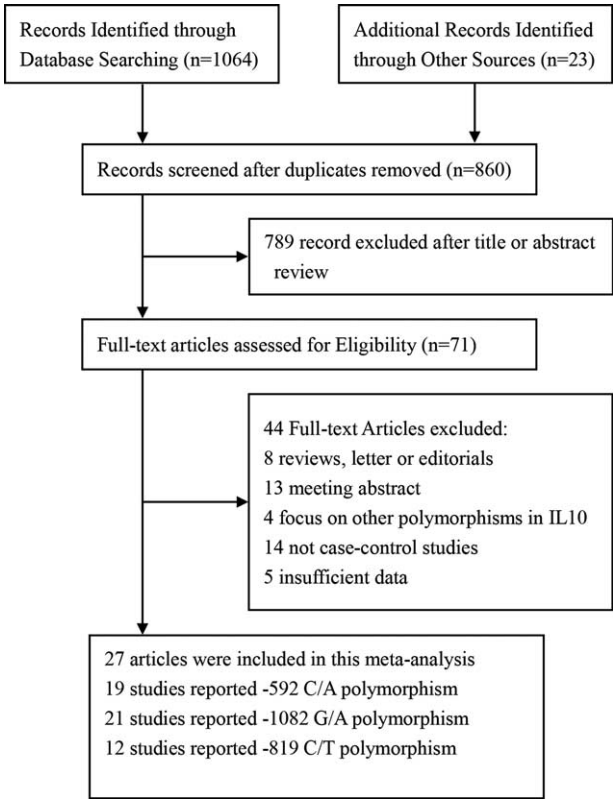

FIGURE 1. Flow chart of the search strategy and study selection. The terms ''N'' in the boxes represent the number of corresponding studies.

Association Between the *IL-10* Polymorphisms and CVD Risk

Data on *IL-10* -592C/A polymorphism were obtained from 19 studies including 7284 CVD patients and 7469 controls.[10,13–17,19–29,32] In overall comparison, there was no obvious evidence of an association between *IL-10* -592C/A polymorphism and the incidence of CVD under all genetic models (A vs C: OR = 1.03, 95% CI = 0.90–1.17; AA vs CC: OR = 1.08, 95% CI = 0.82–1.41; AC vs CC: OR = 1.04, 95% CI = 0.88–1.22; AA vs AC + CC: OR = 1.00, 95% CI = 0.83–1.20; AA +AC vs CC: OR = 1.06, 95% CI = 0.88–1.26) (Figure 2A and Table 3). Similar results were identified in subgroup analysis in light of ethnicity, disease subtype, and quality score. Significant between-study heterogeneity was observed under all 5 genetic models. In the subgroup analysis, heterogeneity vanished in stroke studies as well as dramatically decreased in white subgroup (Table 3).

TSA showed that 39.6% (14753) of the required information size of 37,263 subjects were accrued. The cumulative *Z*-curve has not crossed the conventional boundary before reaching the required information size, suggesting that there was insufficient evidence to show a 6% relative risk increase, and further studies are necessary (Supplemental Figure 1, http://links.lww.com/MD/A696). The TSA-adjusted 95% CI was 0.78 to 1.44.

Twenty-one studies had data on *IL-10* -1082G/A polymorphism, with 8263 CVD patients and 5765 controls.[4,10,14–20,22,24,26,30–32,44–48] Likewise, we failed to confirm the association between *IL-10* -1082G/A polymorphism and CVD risk under all genetic models (A vs G: OR = 1.03, 95% CI = 0.91–1.16; AA vs GG: OR = 1.02, 95% CI = 0.81–1.29; AG vs GG: OR = 0.94, 95% CI = 0.78–1.15; AA vs AG + GG: OR = 1.08, 95% CI = 0.90–1.31; AA + AG vs GG: OR = 0.98, 95% CI = 0.82–1.19) (Figure 2B and Table 3). In the subgroup according to ethnicity, disease subtype and quality score, similar trends with overall results were observed. Substantial heterogeneities were noticed under all 5 genetic models.

TABLE 1. Characteristics of the Eligible Studies Included in This Meta-analysis

First Author	Year	Country	Ethnicity	Outcome	Matching	Genotyping Method	Sample Size (Case/Control)	Age, y		Male, %		NOS Score
								Case	Control	Case	Control	
Balding et al[21]	2004	Ireland	White	IS	NA	PCR	105/389	69 (35–99)	37.1 (18–65)	60	58	6
Zhang et al[16]	2007	China	Asian	CI	NA	PCR-RFLP	204/131	55 ± 9	35 ± 5	59.3	68.7	6
Tuttolomondo et al[31]	2012	Italy	White	IS	Age	ASO-PCR	96/48	71.9 ± 9.75	71.4 ± 7.45	46.9	33.3	7
Sultana et al[48]	2011	Indian	Asian	IS	NA	ARMS-PCR	238/226	53.72 ± 11.11	54.06 ± 10.98	68.9	53.5	7
Seifart et al[30]	2005	Germany	White	CHD	NA	PCR-RFLP	104/243	NA	37.9	NA	55.0	5
Qi et al[28]	2014	China	Asian	IS	Age, sex	MALDI-TOF MS	426/426	46.4 ± 10.5	43.7 ± 9.7	58.7	55.5	8
Marousi et al[18]	2011	Greece	White	IS	Age, sex	RT-PCR	145/145	68 (58–76)	69 (58–77)	65.5	65.5	9
Munshi et al[4]	2010	Indian	Asian	IS	Age, sex	ARMS-PCR	480/470	49.3 ± 17.34	47.01 ±16.78	74.4	74.7	9
Jin et al[25]	2013	China	Asian	AP, AMI	NA	MALDI-TOF MS	249/132	65.85 ± 9.85	63.60 ± 9.05	70.3	61.4	5
Fragoso et al[15]	2011	Mexico	Mixed	MI, UA	Age, sex	TaqMan	389/302	59 (52–66)	55 (50–61)	90	74	8
Elsaid et al[44]	2014	Egypt	White	IHD	NA	PCR	108/143	53.54 ± 9.1	45.3 ± 7.2	67.6	NA	4
Yu et al[32]	2012	Korea	Asian	MI, AP	NA	pyrosequencing	173/313	61.64 ± 9.83	61.37 ± 12.58	67.1	37.4	6
Nasibullin et al[27]	2014	Russia	White	MI	NA	PCR	225/257	45.8 ± 5.27	43.04 ± 7.17	100	100	6
Zuo et al[13]	2014	China	Asian	MI, UA	Age, sex	TaqMan	212/218	NA	NA	77.4	78.9	7
Babu et al[17]	2012	India	Asian	ACS	Age, sex	ARMS-PCR	651/432	53.56 ± 11.72	52.62 ± 8.45	76.66	61.34	7
Karaca et al[19]	2011	Turkey	White	CHD	Age	PCR-RFLP	86/88	43.36 ± 4.93	47.07 ± 8.14	69.8	71.6	7
Ben-Hadj-Khalifa et al[22]	2010	Tunisia	White	CAD	Age, sex	AS-PCR	291/291	56.7 ± 12.2	56.3 ± 12.5	75.6	75.6	8
Lorenzova et al[46]	2007	Czech Republic	White	AMI	Age	PCR-RFLP	284/568	54.1 ± 6.8	adult	100	100	7
O'Halloran et al[47]	2006	Ireland	White	ACS, SA	NA	AS-PCR	1598/386	NA	NA	77.1	58.3	6
Afzal et al[20]	2012	Pakistan	Asian	CAD	Age	ARMS-PCR	93/99	NA	NA	NA	NA	7
Rosner et al[29]	2005	America	White	MI	Age, current smoking status	PCR	522/2089	58.7 ± 8.6	58.8 ± 8.5	100	100	8
Koch et al[14]	2001	Germany	White	CAD, MI	Age, sex	AS-PCR	1791/340	CAD 64.1 ± 10.2 MI 62.6 ± 11.6	63.4 ± 10.3	CAD 75.9 MI 77.4	75.3	7
Donger et al[10]	2001	France, United Kingdom	White	MI	Age, sex	PCR-SSCP	1107/1082	NA	NA	NA	NA	8
Lio et al_a[26]	2004	North Italy	White	AMI	Age	PCR-SSP	142/153	67 (55–80)	67 (65–73)	100	100	7
Lio et al_b[26]	2004	South Italy	White	AMI	Age	PCR-SSP	90/110	41 (23–46)	38 (20–55)	100	100	7
Ianni et al[45]	2012	North Italy	White	AMI	NA	TaqMan	267/321	67.7 ± 12.2	72.0 ± 5.1	73.0	49.2	6
Biswas et al[23]	2014	India	Asian	MI	Age, sex	AS-PCR	500/500	40–65	NA	91.6	91.6	7
Cruz et al[24]	2013	Mexico	Mixed	SMI	NA	TaqMan	149/248	61 ± 8.31	56.0 ± 4.12	38.9	79.0	6

ACS = acute coronary syndrome, AMI = acute myocardial infarction, AP = angina pectoris, ARMS-PCR = amplification refractory mutation system-PCR, ASO-PCR = allele specific oligonucleotides-PCR, AS-PCR = allele specific-PCR, CAD = coronary artery disease, CHD = coronary heart disease, CI = cerebral infarction, HWE = Hardy-Weinberg equilibrium, IHD = ischemic heart disease, IS = ischemic stroke, MALDI-TOF MS = matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, MI = myocardial infarction, NA = not available, NOS = Newcastle Ottawa Scale, PCR = polymerase chain reaction, PCR-RFLP = PCR-restriction fragment length polymorphism, PCR-SSCP = PCR-single strand conformation polymorphism, PCR-SSP = PCR-sequence specific primer, RT-PCR = real time-PCR, SA = stable angina, SMI = silent myocardial ischemia, UA = unstable angina.

TABLE 2. Genotype Distribution Among Studies Included in the Meta-analysis

First Author	Year	−592C/A (Case/Control)				−1082G/A (Case/Control)				−819C/T (Case/Control)			
		AA	AC	CC	HWE (*P*)	AA	AG	GG	HWE (*P*)	TT	CT	CC	HWE (*P*)
Balding et al[21]	2004	5/15	35/139	65/235	0.317								
Zhang et al[16]	2007	86/56	90/48	28/27	0.009	202/120	2/11	0/0	0.616	86/56	90/48	28/27	0.009
Tuttolomondo et al[31]	2012					58/20	14/17	24/11	0.066	19/5	14/17	63/26	0.390
Sultana et al[48]	2011					154/163	44/47	40/16	<0.001				
Seifart et al[30]	2005					19/86	59/115	25/42	0.739	5/14	25/88	74/140	0.972
Qi et al[28]	2014	199/193	172/167	55/66	0.004								
Marousi et al[18]	2011					47/53	71/71	27/21	0.723				
Munshi et al[4]	2010					92/63	241/218	147/189	0.991				
Jin et al[25]	2013	134/61	99/52	16/19	0.156								
Fragoso et al[15]	2011	67/81	179/139	143/82	0.167	211/164	142/113	36/25	0.380	61/56	175/146	153/100	0.833
Elsaid et al[44]	2014					2/8	49/85	22/5	<0.001				
Yu et al[32]	2012	76/172	80/117	17/24	0.511	150/275	22/38	1/0	0.253	76/167	80/125	17/21	0.712
Nasibullin et al[27]	2014	12/13	77/98	136/146	0.505								
Zuo et al[13]	2014	126/104	71/90	15/24	0.499								
Babu et al[17]	2012	77/74	253/228	321/130	0.126	318/170	260/188	73/74	0.079				
Karaca et al[19]	2011	6/6	29/24	51/58	0.129	20/21	44/44	22/23	0.996				
Ben-Hadj-Khalifa et al[22]	2010	25/16	109/83	156/188	0.099	76/52	108/100	101/76	0.088	10/8	87/80	191/162	0.620
Lorenzova et al[46]	2007					90/207	98/255	40/106	0.083				
O'Halloran et al[47]	2006					324/77	784/138	490/117	0.004				
Afzal et al[20]	2012	5/15	84/81	4/3	<0.001	6/4	77/92	10/3	<0.001	5/15	84/81	4/3	<0.001
Rosner et al[29]	2005	37/136	176/720	309/1233	0.028								
Koch et al[14]	2001	114/27	684/138	993/175	0.977	540/105	874/161	377/74	0.407	114/27	684/138	993/175	0.977
Donger et al[10]	2001	33/42	342/337	612/576	0.408	242/231	486/477	256/244	0.944	32/41	340/337	611/576	0.344
Lio et al_a[26]	2004	14/8	43/44	85/101	0.277	60/30	52/75	30/48	0.942	14/8	43/44	85/101	0.277
Lio et al_b[26]	2004	9/8	31/36	50/66	0.327	44/28	29/56	17/26	0.846	9/8	31/36	50/66	0.327
Ianni et al[45]	2012					68/78	141/88	56/73	<0.001				
Biswas et al[23]	2014	142/104	248/252	110/144	0.746								
Cruz et al[24]	2013	28/41	72/113	49/94	0.478	55/125	83/106	11/17	0.387	28/44	69/119	52/85	0.833

HWE = Hardy-Weinberg equilibrium.

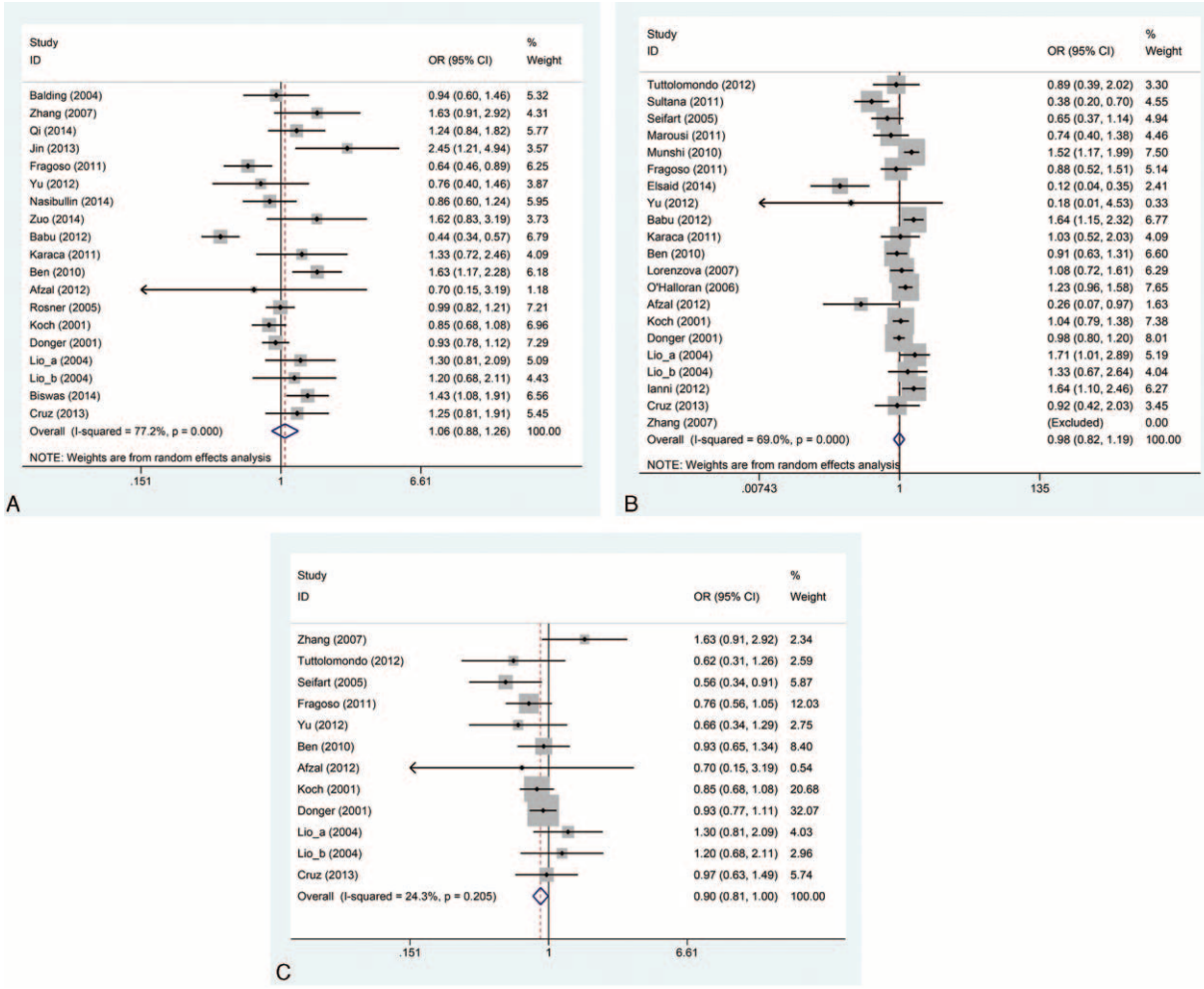

FIGURE 2. Forest plot of the risk of CVD associated with the *IL-10* gene polymorphisms under dominant genetic model. (A) *IL-10*-592C/A polymorphism. (B) *IL-10*-1082G/A polymorphism. (C) *IL-10*-819C/T polymorphism. The solid diamonds and horizontal lines correspond to the study-specific ORs and 95% 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. CI = confidence interval, CVD = cardiovascular disease, OR = odds ratio.

Following subgroup analysis, heterogeneities almost disappeared in mixed population subgroup (Table 3).

TSA showed that 13,693 of the required information size of 89,658 subjects were accrued. The required information size is far from reached and the conventional boundary has not been crossed, leaving the meta-analysis inconclusive of a 2% relative risk reduction (Supplemental Figure 2, http://links.lww.com/MD/A696). The TSA-adjusted 95% CI was 0.46 to 2.11.

Twelve studies provided data on *IL-10* −819C/T polymorphism consisting of 4502 CVD cases and 3190 controls.[10,14–16,20,22,24,26,30–32] Overall, the pooled results revealed a significant association between *IL-10* -819C/T polymorphism and decreased CVD risk under allelic comparison and dominant model (T vs C: OR = 0.91, 95% CI = 0.84–0.98; TT + TC vs CC: OR = 0.90, 95% CI = 0.81–1.00) (Figure 2C and Table 3). If we set $\alpha = 0.05$, based on the data set for -819 T allele, we have a 77.2% power to detect an OR of 0.91. Similar results were found when the meta-analysis was restricted to studies whose controls were in agreement with HWE. In the subgroup analysis stratified by disease subtype, the statistically significant association were found for CAD under all genetic models except heterozygote comparison (T vs C: OR = 0.90, 95% CI = 0.83–0.97; TT vs CC: OR = 0.81, 95% CI = 0.66–1.00; TT vs TC + CC: OR = 0.82, 95% CI = 0.69–0.98; TT + TC vs CC: OR = 0.89, 95% CI = 0.80–0.99) (Table 3). No significant heterogeneity was detected under any genetic models. Meanwhile, no obvious heterogeneity was observed in the vast majority of subgroups.

Using the TSA, the required information size is 4495 subjects to demonstrate the issue. Until now, the cumulative *Z*-curve has crossed the conventional boundary and the required information size has been reached, confirming that *IL-10* -819 C/T polymorphism is associated with decreased risk of CVD and further relevant trials are unnecessary (Figure 3). The TSA adjusted 95% CI was 0.80 to 1.00. Additionally, the TSA of 10 studies reporting CAD showed that sufficient evidence was established to show a relative risk reduction of 11%, the cumulative *Z*-curve has crossed the conventional boundary and the required information size has been reached (Figure 4). The TSA adjusted 95% CI was 0.78 to 1.01. The FPRP values for all

TABLE 3. Meta-analysis of the Association Between 3 *IL-10* Gene Polymorphisms and CVD risk

		Allele		Homozygous		Heterozygous		Recessive		Dominant	
Variables	**Case/Control**	**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	7284/7469	1.03 (0.90–1.17)	<0.001	1.08 (0.82–1.41)	<0.001	1.04 (0.88–1.22)	<0.001	1.00 (0.83–1.20)	<0.001	1.06 (0.88–1.26)	<0.001
Overall (controls in HWE)	6039/4724	1.03 (0.87–1.22)	<0.001	1.08 (0.77–1.51)	<0.001	1.02 (0.83–1.24)	<0.001	1.03 (0.82–1.30)	<0.001	1.04 (0.84–1.29)	<0.001
Ethnicity											
White	4238/4668	1.04 (0.92–1.18)	0.041	1.04 (0.84–1.27)	0.261	1.00 (0.90–1.10)	0.252	1.03 (0.84–1.26)	0.471	1.03 (0.90–1.19)	0.089
Asian	2508/2251	1.02 (0.79–1.34)	<0.001	1.11 (0.67–1.85)	<0.001	1.13 (0.72–1.78)	<0.001	0.99 (0.74–1.32)	<0.001	1.14 (0.70–1.85)	<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
Disease subtype											
CAD	6549/6523	1.02 (0.87–1.19)	<0.001	1.04 (0.76–1.42)	<0.001	1.01 (0.84–1.21)	<0.001	0.99 (0.79–1.24)	<0.001	1.03 (0.84–1.26)	<0.001
Stroke	735/946	1.08 (0.93–1.27)	0.826	1.30 (0.94–1.79)	0.886	1.19 (0.90–1.57)	0.223	1.04 (0.83–1.31)	0.900	1.19 (0.92–1.54)	0.322
Quality score											
High	6179/5999	1.02 (0.87–1.21)	<0.001	1.01 (0.72–1.42)	<0.001	0.99 (0.82–1.21)	<0.001	0.99 (0.78–1.27)	<0.001	1.02 (0.82–1.27)	<0.001
Low	1105/1470	1.03 (0.85–1.26)	0.039	1.26 (0.94–1.68)	0.128	1.10 (0.89–1.35)	0.116	0.97 (0.79–1.19)	0.166	1.15 (0.85–1.55)	0.061
IL-10 -1082G/A		A vs G		AA vs GG		AG vs GG		AA vs AG + GG		AA + AG vs GG	
Overall	8263/5765	1.03 (0.91–1.16)	<0.001	1.02 (0.81–1.29)	<0.001	0.94 (0.78–1.15)	<0.001	1.08 (0.90–1.31)	<0.001	0.98 (0.82–1.19)	<0.001
Overall (controls in HWE)	5996/4771	1.10 (0.96–1.27)	<0.001	1.16 (0.91–1.49)	<0.001	1.04 (0.93–1.16)	0.306	1.19 (0.96–1.47)	<0.001	1.09 (0.94–1.27)	0.057
Ethnicity											
White	5886/3544	1.04 (0.90–1.19)	<0.001	1.05 (0.82–1.35)	<0.001	0.95 (0.76–1.19)	<0.001	1.08 (0.85–1.37)	<0.001	1.01 (0.83–1.23)	0.002
Asian	1839/1671	1.10 (0.78–1.55)	<0.001	0.94 (0.43–2.04)	<0.001	0.78 (0.42–1.44)	0.001	1.29 (0.87–1.93)	0.002	0.79 (0.41–1.56)	<0.001
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
Disease subtype											
CAD	7100/4745	1.02 (0.90–1.16)	<0.001	1.06 (0.83–1.35)	<0.001	1.00 (0.82–1.22)	<0.001	1.04 (0.85–1.27)	<0.001	1.03 (0.85–1.25)	<0.001
Stroke	1163/1020	1.16 (0.73–1.84)	<0.001	0.91 (0.40–2.03)	<0.001	0.68 (0.32–1.41)	0.001	1.38 (0.80–2.38)	<0.001	0.81 (0.40–1.60)	<0.001
Quality score											
High	5698/4161	1.11 (0.98–1.25)	<0.001	1.18 (0.93–1.49)	<0.001	0.98 (0.81–1.20)	0.001	1.20 (1.00–1.45)	<0.001	1.07 (0.89–1.29)	<0.001
Low	2565/1604	0.82 (0.59–1.15)	<0.001	0.46 (0.21–1.00)	0.004	0.69 (0.33–1.46)	<0.001	0.75 (0.48–1.20)	0.002	0.58 (0.28–1.23)	<0.001
IL-10 -819C/T		T vs C		TT vs CC		TC vs CC		TT vs TC + CC		TT + TC vs CC	
Overall	4502/3190	**0.91 (0.84–0.98)**	0.215	0.88 (0.73–1.06)	0.219	0.90 (0.81–1.00)	0.131	0.86 (0.74–1.01)	0.249	**0.90 (0.81–1.00)**	0.205
Overall (controls in HWE)	4205/2960	**0.90 (0.83–0.98)**	0.217	0.85 (0.69–1.03)	0.389	**0.88 (0.79–0.98)**	0.244	0.88 (0.73–1.04)	0.352	**0.88 (0.79–0.98)**	0.331
Ethnicity											
White	3494/2097	0.93 (0.84–1.02)	0.194	0.91 (0.70–1.19)	0.328	0.86 (0.71–1.05)	0.096	0.96 (0.75–1.25)	0.298	0.90 (0.80–1.01)	0.210
Asian	470/543	0.88 (0.67–1.16)	0.123	0.74 (0.30–1.82)	0.047	1.15 (0.62–2.13)	0.193	0.71 (0.45–1.11)	0.128	0.99 (0.50–1.98)	0.114
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)	0.508	0.90 (0.66–1.24)	0.418	0.83 (0.65–1.07)	0.371
Disease subtype											
CAD	4202/3011	**0.90 (0.83–0.97)**	0.202	**0.81 (0.66–1.00)**	0.362	0.89 (0.80–1.00)	0.689	**0.82 (0.69–0.98)**	0.323	**0.89 (0.80–0.99)**	0.404
Stroke	300/179	1.09 (0.83–1.44)	0.564	1.50 (0.87–2.59)	0.929	0.80 (0.16–4.13)	0.002	1.11 (0.74–1.67)	0.182	1.03 (0.40–2.65)	0.038
Quality score											
High	3872/2256	0.92 (0.84–1.00)	0.409	0.85 (0.67–1.07)	0.231	0.90 (0.80–1.02)	0.353	0.88 (0.71–1.09)	0.127	0.90 (0.81–1.01)	0.548
Low	630/934	0.88 (0.68–1.12)	0.072	0.95 (0.68–1.34)	0.203	0.91 (0.56–1.47)	0.036	0.85 (0.66–1.08)	0.490	0.87 (0.56–1.37)	0.036

CAD = coronary artery disease, CI = confidence interval, HWE = Hardy-Weinberg equilibrium, OR = odds ratio, P^{het} = P value for heterogeneity test. The OR values with statistical significance were shown in bold.

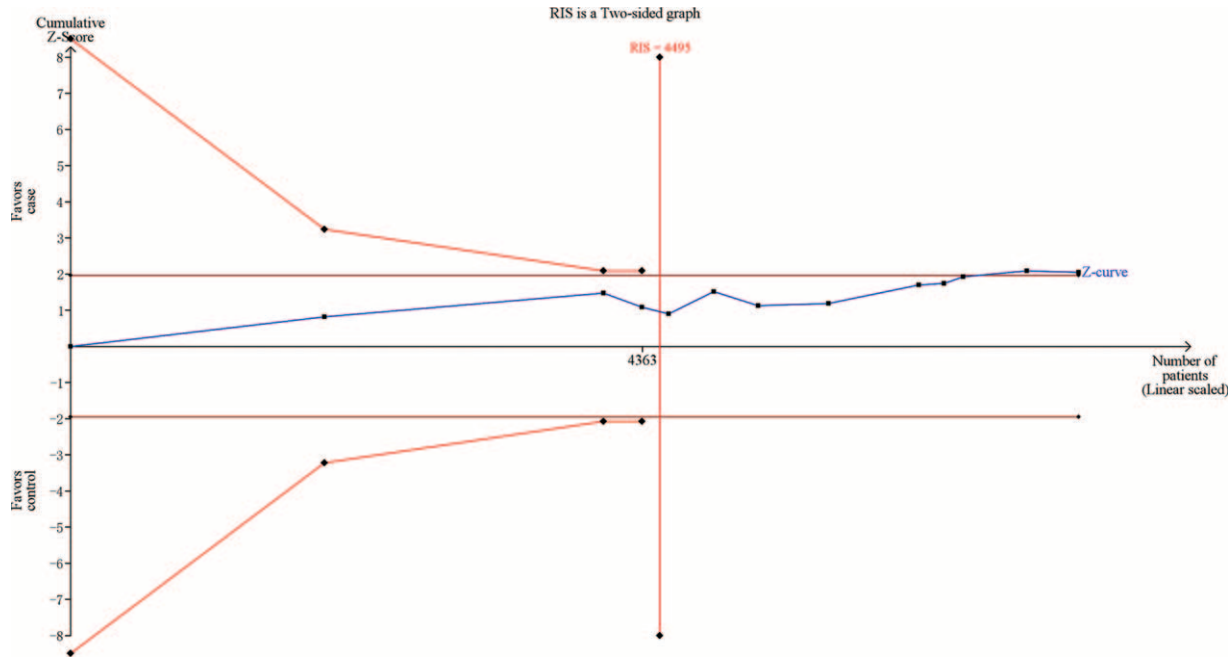

FIGURE 3. Trial sequential analysis of 12 studies reporting *IL-10* -819C/T polymorphism. The required information size was calculated using $\alpha = 0.05$ (2-sided), $\beta = 0.20$ (power 80%), $D^2 = 39\%$, a relative risk reduction of 10% and an event proportion of 53.5% in the control arm. The blue cumulative *Z*-curve was constructed using a fixed-effects model.

significant findings are shown in Supplemental Table 2, http://links.lww.com/MD/A696. For a prior probability of 0.1 and OR of 0.67, the FPRP analyses suggested that all significant associations were deserving of attention.

The Correlation Between the mRNA Expression and Genotypes

The correlation between *IL10* mRNA expressions levels by the genotypes were explored for all population (Supplemental

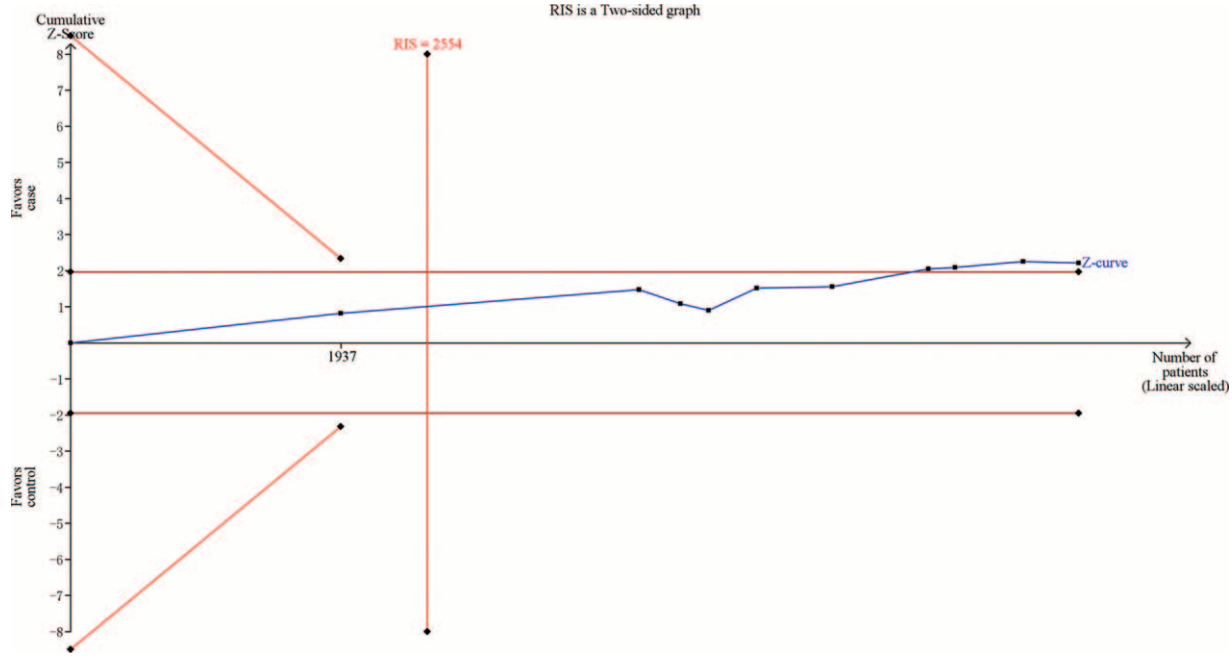

FIGURE 4. Trial sequential analysis of 10 studies reporting the association between *IL-10* -819C/T polymorphism and CAD. The required information size was calculated using $\alpha = 0.05$ (two sided), $\beta = 0.20$ (power 80%), $D^2 = 8\%$, a relative risk reduction of 11% and an event proportion of 52.54% in the control arm. The blue cumulative *Z*-curve was constructed using a fixed-effects model. CAD = coronary artery disease.

Figure 3, http://links.lww.com/MD/A696). No significant alteration in the mRNA expression levels was found for the 3 variants.

Sensitivity Analysis and Publication Bias

Sensitivity analyses were performed to assess the influence of each individual study on the pooled OR in each comparison in the polymorphisms of *IL-10* -592C/A, *IL-10* -819C/T, and *IL-10* -1082G/A. The recalculated ORs were not significantly influenced, which suggested our results were robust and reliable. Begg funnel plot and Egger test were performed to evaluate the potential publication bias of literatures. The shapes of the funnel plots showed no evidence of obvious asymmetry (Figure 5). The Egger test results did not support the existence of publication bias. The $Nfs_{0.05}$ values for *IL-10* -592C/A, *IL-10* -1082G/A, and *IL-10* -819C/T polymorphisms were 242, 208, 51, respectively, which were consistently greater than the number of studies included in this meta-analysis.

DISCUSSION

It is now accepted that inflammation play a significant role in the pathophysiology of CVD.[4,5] IL-10 is a potent anti-inflammatory cytokine with multiple functions taking part in inflammation reaction as well as the development of CVD.[4,11,12] Recently, the associations between 3 *IL-10* gene polymorphisms and the risk of CVD have been intensively investigated; however, the results are inconsistent. Thus, we conducted a systematic review with meta-analysis and TSA to obtain a more precise conclusion.

Although data from some individual studies suggested a relationship, the overall result of the present meta-analysis argued against an association of *IL-10* -592C/A or *IL-10* -1082G/A polymorphism with CVD risk in all genetic models. We also performed genotype-based mRNA expression analysis using the data from 270 individual. The biological results are in accordance with the observed association. Moreover, further sub-analysis of either gene polymorphism based on ethnicity, disease subtype, or quality score did not suggest a significantly different result. There are 3 potential reasons for the results. First, because of the complex nature of CVD, it is unlikely that a SNP in a single gene would be associated with an increased risk of CVD, without a contribution from other polymorphic susceptibility genes. Second, *IL-10* -592C/A or *IL-10* -1082G/A polymorphism itself might exhibit null contribution to the susceptibility of CVD. Third, other factors, such as age, medical treatment, and nutrient status, can also influence the risk of CVD. However, TSA did not allow us to draw any solid conclusion on the association between *IL-10* -592C/A or *IL-10* -1082G/A polymorphism and CVD risk. Thus, these issues need to be further studied.

As for *IL-10* -819C/T polymorphism, our result showed that the individuals who carry the T allele have 10% decreased risk of CVD compared with the CC homozygote carriers, and a

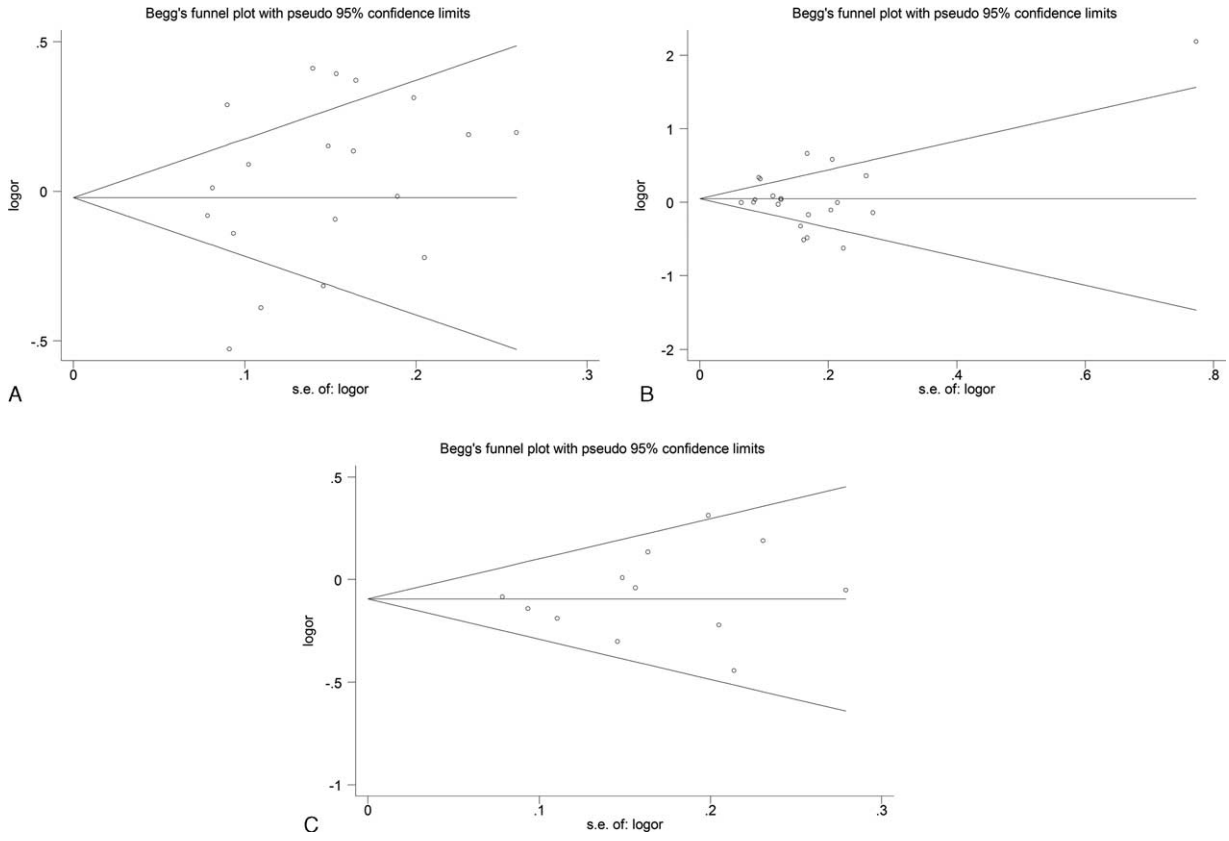

FIGURE 5. Begg funnel plot of publication bias in the meta-analysis of the association of *IL-10* gene polymorphisms with CVD risk under allele genetic model. (A) *IL-10* -592C/A polymorphism. (B) *IL-10* -1082G/A polymorphism. (C) *IL-10* -819C/T polymorphism. Each circle represents a separate study for the indicated association. CVD = cardiovascular disease, logor = natural logarithm of OR; s.e. = standard error.

significantly decreased risk of CVD was also found in allele model. This may be because of the fact that *IL-10* -819 T allele potently alters the *IL-10* gene activity resulting in a marked increase of plasma IL-10 concentration. Moreover, TSA provided firm evidence of -819C/T polymorphism associated with decreased risk of CVD. In the subgroup analysis stratified by disease subtype, we discovered that -819C/T polymorphism had a significant correlation with CAD in all genetic models except heterozygous model. The result of TSA suggested evidence was sufficient enough for this relationship. However, in stroke subgroup, the data were obtained only from two studies. So, the findings in this subgroup should be interpreted with caution.

To make the conclusion more credible, we performed the FPRP analysis, publication bias analysis and sensitivity analysis. All significant associations passed the FPRP analyses, indicating that these associations were robust. Funnel plots suggested that no obvious publication bias was detected. The $N_{fs0.05}$ for 3 polymorphisms were greater than the number of studies included in this meta-analysis, also indicating a low probability of publication bias. The sensitivity analysis revealed that the results are robust and no single study could alter the pooled ORs obviously.

For meta-analysis, the existence of heterogeneity among the available studies affects the reliability of the results in a large extent. Thus, we defined a limited number of potential heterogeneity factors before performing our meta-analysis. As for *IL-10* -592C/A polymorphism, results from subgroup analysis suggested that the ethnicity and disease subtype might be the sources of heterogeneity. Regarding *IL-10* -1082G/A polymorphism, the ethnicity might contribute to the between-study heterogeneity.

As far as we know, this is the first comprehensive meta-analysis exploring the association between 3 *IL-10* gene polymorphisms and CVD risk up to now. Previous meta-analysis mainly focused on *IL-10* -1082G/A polymorphism and stroke risk,[49–52] whereas our meta-analysis included more studies concerning 3 well-characterized polymorphisms in the *IL-10* gene and two CVD outcomes (CAD and stroke). Our meta-analysis also has some advantages. First, the search and selection studies were conducted strictly. Second, no evidence of publication bias was found by Begg funnel plot and Egger test. Third, TSA was performed, which could reduce the type I error rate. In addition, we performed false-positive report probability analysis to preclude false association resulting from multiple calculations.

Despite the clear strengths of this meta-analysis, including the large sample size and the implementation of TSA, several limitations should be addressed. First, the included studies were published in English and Chinese, whereas studies published in other languages were ignored. Second, there was significant heterogeneity in some of the pooled analysis, which may have affected the meta-analysis results even though we adopted the random-effects model. Third, several studies deviate from HWE expectations. Though, when the analysis was restricted to the studies in HWE, the pooled results did not alter significantly. Fourth, 2 studies in our meta-analysis included population with Latinos, and we did not find studies performed in other mixed ethnicities, so it is hard to make a definite conclusion about the population-specific genetic differences between the 3 polymorphisms and CVD risk; further studies should pay attention to the ethnic-specific effects on CVD susceptibility.

In conventional meta-analyses, *IL-10* -592C/A and *IL-10* -1082G/A polymorphisms were not likely to exert any influence on the susceptibility of CVD, whereas the *IL-10* -819C/T polymorphism might be a protective factor for CVD, especially for CAD outcome, suggesting potential implications for genotyping the *IL-10* -819C/T polymorphism in CAD risk appraisal. After TSA adjustment for sparse data and multiple updating in cumulative meta-analysis, it seems unsure that *IL-10* -592C/A and *IL-10* -1082G/A polymorphisms were not associated with the risk of CVD. Considering our main limitations, larger well-designed studies are necessary. Moreover, other IL polymorphisms and gene–gene interactions should also be considered in future studies.

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