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Gene polymorphisms and circulating levels of the TNF-alpha are associated with ischemic stroke: A meta-analysis based on 19,873 individuals

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ABSTRACT

Objective: Numerous studies have investigated associations of gene polymorphisms and circulating levels of TNF-α with ischemic stroke (IS), but the results were controversial. The aims of this study were to systematically evaluate these associations.

Methods: Relevant publications were retrieved by searching databases. Odds ratios (ORs) and standard mean differences (SMDs) with 95% confidence intervals (95% CIs) were used to assess the association of the TNF-α gene and cytokine with IS, respectively. The Cochrane Q test and I^2 statistic were used to test heterogeneity. Subgroup analysis and publication bias were performed.

Results: 25 and 9 articles examined the association of polymorphisms and levels of the TNF-α with IS risk, respectively. Rs1800629 polymorphism was associated with IS susceptibility (OR (95% CI) = 0.82 (0.72, 0.95)), especially in Asians (OR (95% CI) = 0.75 (0.63, 0.89)); and rs1800610 was associated with IS susceptibility in Asians patients (OR (95% CI) = 1.54 (1.31, 1.80)). While rs361525, rs1799964 and rs1799724 polymorphisms were not associated with IS susceptibility. The TNF-α level was elevated in IS patients (SMD (95% CI) = 0.65 (0.29, 1.01)) including Asians (SMD (95% CI) = 1.26 (0.49, 2.03)) and Caucasians (SMD (95% CI) = 0.26 (0.03, 0.49)). In addition, increased level occurred in patients' serum (SMD (95% CI) = 0.54 (0.08, 1.01)).

Conclusions: Rs1800629 and rs1800610 polymorphisms were elucidated to be a protective factor for IS (especially in Asians) and a risk factor for Asians patients, respectively. The TNF-α level was elevated in IS, indicating that TNF-α plays an important role in the pathogenesis of IS and is a promising therapeutic target for IS.

1. Introduction

Ischemic stroke (IS) is a major cause of mortality and morbidity in adults [1,2], which is the results of interaction of genetic variations and environmental factors [3]. Numerous studies have found that inflammation and pro-inflammatory cytokines were involved in the pathogenesis of stroke [4,5]. Therefore, inflammatory gene polymorphisms and circulating cytokine levels may be associated with the incidence and outcome of IS.

Tumor necrosis factor-α (TNF-α) is the most important pro-inflammatory cytokine that has protective and destructive effects on the central nervous system [6,7]. Some studies from different countries have found that the single nucleotide polymorphisms (SNPs) of the TNF-α gene were related to the susceptibility of IS, but the results were controversial. Cui et al. [8] found that IS susceptibility was associated with the rs1800629 but not associated with rs361525. Their results were opposite to those of Liamas and colleagues [9]. Furthermore, the opposite results of the rs1799964 occurred in the two phases of one study [8]. Therefore, the role of TNF-α gene in the pathogenesis of IS needs to be further elucidated. In addition, the TNF-α gene promoter

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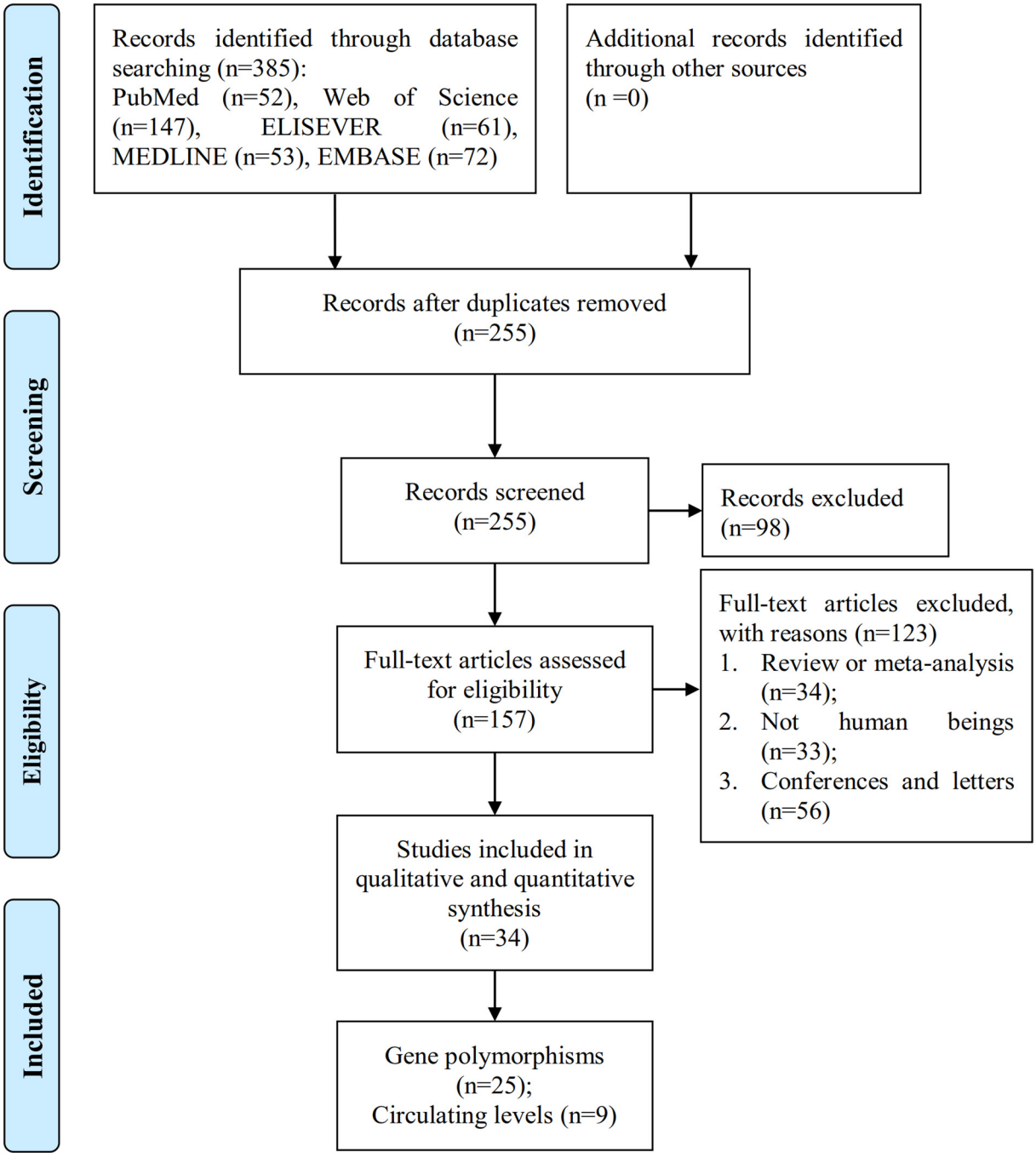

Fig. 1. Flow chart.

polymorphisms were associated with an increase of TNF-α cytokine [10]. Elevated circulating level of TNF-α plays an important role in coagulation, lipid metabolism and endothelial function, and may increase the risk of IS [11,12]. Patients with IS had a higher level of TNF-α compared with controls [13–15], but some studies failed to find these results [16,17].

The purpose of this meta-analysis was to assess the association of the TNF-α gene polymorphisms and cytokine levels with the risk of IS by synthesizing the best available evidence from published studies.

2. Materials and methods

2.1. Search strategy

This systematic review was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [18]. PubMed, Web of Science, MEDLINE, EMBASE and ELISWER databases were retrieved without the publication year restrictions until April 2019. Keywords included “cerebrovascular disease” or “cerebrovascular disorder” or “brain infarction” or “ischemic stroke” or “stroke”, “TNF” or “tumor necrosis factor” and “human”. We retrieved eligible studies and hand-searched their references. Two authors (Jun-cang Wu and Xu Zhang) conducted the literature search independently and discussed with the third author (Sen Qun) when there were disagreements.

2.2. Inclusion criteria and exclusion criteria

Inclusion criteria: a) studies investigated the associations of polymorphisms and circulating levels of TNF-α with IS; b) studies provided data on genotype distributions or circulating levels of TNF-α in case and control groups; c) studies performed in humans and published in English; d) patients without other cardiovascular diseases (*e.g.*, atherosclerosis obliterans); e) controls without cerebrovascular disease. Exclusion criteria: a) non-compliance with inclusion criteria; b) *in vivo* and *in vitro* studies; c) review, meta-analysis, conference, and case-report articles.

2.3. Quality assessment and data extraction

Two investigators evaluated the quality of the included studies according to the Newcastle–Ottawa quality assessment scale (NOS). The following items of each study were extracted, including first author's name, publication year, ethnicity, sample size, genotype frequencies of the TNF-α gene in two groups, circulating level of the TNF-α, and source of specimen. If the above data were not available, we sent an e-mail to the author for requesting the full-text.

2.4. Statistical analysis

Association of the TNF-α gene polymorphisms and levels with IS risk was assessed by odds ratio (OR) with 95% confidence interval (95% CI) and standardized mean difference (SMD) with 95% CI, respectively. When articles reported results using the median (M) and quartiles (P_{25}, P_{75}), we used an approximation method to convert them to mean and standard deviation ($\bar{X} \pm S$) [19]: $\overline{X} \approx \frac{P25 + M + P75}{3}$, $S = \frac{P75 - P25}{1.35}$. Q-statistic and I^2 tests were used to assess heterogeneity, and a random-effects model was used to calculate the pooled OR/SMD when there was a significant heterogeneity ($P < 0.1$ or $I^2 > 40$); otherwise, a fixed-effect model was used. Subgroup analysis was utilized to explore potential sources of heterogeneity: (a) subgroup of ethnicity (gene polymorphisms: Caucasian *vs*. Asian, circulating level: Caucasian *vs*. Asian *vs*. South American), (b) subgroup of specimen source (circulating level: serum *vs*. cerebrospinal fluid *vs*. gingival crevicular fluid *vs*. plasma). Sensitivity analysis was used to assess the effect of one or more studies on overall outcomes. Egger's linear regression and Begg's rank correlation tests were applied for evaluating publication bias, and a trim and fill method was performed if there was a possible publication bias. Statistical analysis was conducted using the STATA 15.0 software (Stata Corp, College Station, TX, USA) and Review Manager 5.1 software (Cochrane Collaboration, Oxford, UK), and a two-side $P < 0.05$ was considered statistically significant.

3. Results

3.1. Study characteristics

We identified 385 potential reports, reviewed 157 full-texts, and included 34 papers in the final analysis (Fig. 1). Of the 34 records, 25 including 8828 IS patients and 9325 controls investigated the association between the TNF-α gene polymorphisms and IS susceptibility [3,8,9,20–39], 9 involving 776 IS patients and 944 controls explored the TNF-α circulating levels [13–15,17,40–44]. The main characteristics and NOS scores of the included studies are shown in Table 1.

3.2. Results of the TNF-α gene polymorphisms

3.2.1. Rs1800629, rs1800610, rs1799964 and IS

A total of 23 studies investigated the association between rs1800629 polymorphism and IS susceptibility. Rs1800629 was associated with IS susceptibility (OR (95% CI) =0.82 (0.70, 0.95), Z = 2.62, $P = 0.009$), especially in Asian patients (OR (95% CI) =0.75 (0.63, 0.89), Z = 3.27, $P = 0.001$), (Table 2 & Fig. 2A). In addition, rs1800610 was associated with IS risk in Asians patients (OR (95% CI) =1.54 (1.31, 1.80), Z = 5.35, $P < 0.001$), (Table 2 & Fig. 2B), but rs1799964 polymorphism was not associated with IS susceptibility (Table 2 & Fig. 2C). There was no publication bias (Table 2).

3.2.2. Rs361525, rs1799724 and IS

There were 11 and 5 articles explored the association of rs361525 and rs1799724 polymorphisms with IS susceptibility, respectively. However, the two SNPs were not associated with IS susceptibility, and there was no publication bias (Table 2 & Fig. 3A–B).

3.3. Results of the TNF-α cytokine

We included 9 articles to investigate the association between TNF-α level and IS. We found the elevated circulating level of TNF-α in IS patients (SMD (95% CI) =0.56 (0.17, 0.94), Z = 2.83, $P = 0.005$), including Asians (SMD (95% CI) =1.26 (0.49, 2.03), Z = 3.19, $P = 0.001$) and Caucasians (SMD (95% CI) =0.26 (0.03, 0.49), Z = 2.23, $P = 0.030$), (Table 2 & Fig. 4A). In addition, the significant difference was also detected in serum samples (SMD (95% CI) =0.54 (0.08, 1.01), Z = 2.29, $P = 0.020$), (Table 2 & Fig. 4B).

There may be publication bias (*P*-value of Egger test) in the overall populations, Caucasians and serum samples, but the trim and fill analyses found no change in results and data (Data not shown), suggesting that our findings are stable.

3.4. Results of sensitivity analysis

Sensitivity analysis showed that the pooled results did not change by omitting the individual study, indicating that the overall effect sizes are robust (Data not shown).

4. Discussion

This meta-analysis explored the relationship between five SNPs of the TNF-α gene and susceptibility of IS and the level of TNF-α cytokine in IS patients was investigated. We found that rs1800610 and rs1800629 SNPs were associated with IS susceptibility, there were no significant differences between IS patients and controls in the remaining three SNPs. In addition, the level of TNF-α cytokine was elevated in IS patients compared with controls. In the subgroup analysis, the rs1800610 and rs1800629 were found to have an effect on Asian populations, and increased level of TNF-α cytokine was found in Asians and Caucasians. In stratification of the specimen, the concentration of TNF-α level was elevated in the serum of IS patients. These findings help us understand the role of gene polymorphisms and cytokine levels of TNF-α in the pathogenesis of IS.

In this study, we found that the rs1800629 SNP can protect against IS risk, especially in Asians, but not in Caucasians. The different outcomes of Asians and Caucasians may be due to the genetic and environmental factors. However, previous meta-analyses reported that the rs1800629 polymorphism was not associated with IS risk, but may be associated with the Asian populations [45,46]. The main reason is that our study enrolled more articles and included a larger number of IS patients and controls, so our results are more convincing. In addition, the statistical power of rs1800629 in overall populations and Asians exceeded 99.9% (Data not shown), suggesting that our results are very stable. While the power of Caucasians was 64.6%, and the synthetic weight of Caucasians was 30.8% (Fig. 1). Therefore, large-scale studies in the future should be conducted in Caucasian populations. Song and Cheng [47] suggested that rs1800629 was not associated with overall populations but associated with the East Asians. One explanation is that individuals from Caucasians and non-East Asians were included in one group [47], but the genetic backgrounds of the two populations are very different, so their analysis is unreasonable. Unlike Song's meta-

Table 1
Characteristics of the included studies.

References	Year	Ethnicity	Sample size		SNP/specimen	NOS
			IS	CTR		
SNP						
Rubattu [20]	2005	Caucasian	115	180	rs1800629	7
Kim [21]	2010	Asian	237	216	rs1800629, rs361525	7
Munshi [22]	2011	Asian	525	500	rs1800610	6
Cui-1 [8]	2012	Asian	1388	1027	rs1799964, rs1800629, rs1799724, rs361525	8
Cui-2 [8]	2012	Asian	961	821	rs1799964, rs1800629, rs1799724, rs361525	8
Llamas [9]	2007	Caucasian	308	302	rs1800629, rs361525	5
Shi [23]	2009	Asian	67	70	rs1799964, rs1800629, rs1799724, rs361525	6
Lee [24]	2004	Asian	152	165	rs1800629	5
Tong-1 [25]	2010	Asian	648	648	rs1800629, rs361525	7
Tong-2 [25]	2010	Asian	100	100	rs1800629, rs361525	7
Wawrzynek [26]	2014	Caucasian	101	100	rs1800629	6
Gu [27]	2016	Asian	616	607	rs1800629, rs361525	6
Karahan [28]	2005	Caucasian	86	83	rs1800629	5
Djordjevic [29]	2013	Caucasian	26	100	rs1800629	5
Kumar [30]	2016	Asian	250	250	rs1799964, rs1800629, rs1799724,rs1800610	6
Harcos [3]	2006	Caucasian	336	333	rs1800629	5
Banerjee [31]	2008	Asian	112	212	rs1800629	6
Lalouschek [32]	2006	Caucasian	404	415	rs1800629, rs361525	6
Um [33]	2004	Asian	366	610	rs1800629	5
Balding [34]	2004	Caucasian	105	389	rs1800629	7
Sultana [39]	2011	Asian	238	226	rs1800629	6
Markoula [35]	2011	Caucasian	173	179	rs1799724	7
Zhao [36]	2012	Asian	1124	1163	rs1800629, rs361525	7
Tuttolomondo [37]	2012	Caucasian	96	48	rs1800629	6
Um [38]	2003	Asian	294	581	rs1800629	6
Cytokine						
Jefferis [40]	2009	Caucasian	299	587	Serum	5
Zaremba [41]	2001	Caucasian	23	15	CSF/serum	6
Wytrykowska [17]	2016	Caucasian	42	34	GSF/serum	7
Cure [42]	2013	Asian	54	50	Serum	5
Zaremba [43]	2001	Caucasian	23	15	CSF/serum	6
Bokhari [13]	2014	Asian	131	47	Plasma	6
Intiso [14]	2004	Caucasian	41	40	Serum	7
Domac [15]	2007	Asian	70	22	Serum	6
Parreira [44]	2015	SA	93	134	Serum	7

CTR, control; CSF, cerebrospinal fluid; GSF, gingival crevicular fluid; IS, ischemic stroke; SA, South America; SNP, single nucleotide polymorphism; NOS, Newcastle–Ottawa quality assessment scale.

Table 2
Results of meta-analysis.

Type	Subgroup	N	OR/SMD (95% CI)	Z	P	Heterogeneity		Model	PB	PE
						I^2%	P			
SNP										
Rs1800629	Caucasian	9	0.97 (0.72, 1.30)	0.22	0.830	70	< 0.001	R	0.917	0.946
	Asian	15	0.75 (0.63, 0.89)	3.27	0.001	65	< 0.001	R	0.198	0.183
	Overall	24	0.82 (0.70, 0.95)	2.62	0.009	68	< 0.001	R	0.535	0.442
Rs1800610	Asian	2	1.54 (1.31, 1.80)	5.35	< 0.001	0	0.620	F	1.000	NA
Rs1799964	Asian	4	0.96 (0.81, 1.14)	0.48	0.630	54	0.090	R	0.734	0.774
Rs361525	Caucasian	2	1.61 (0.90, 2.88)	1.60	0.110	67	0.080	R	1.000	NA
	Asian	9	0.99 (0.86, 1.15)	0.10	0.920	0	0.980	F	0.917	0.997
	Overall	11	1.10 (0.96, 1.26)	1.40	0.160	31	0.150	F	0.533	0.960
Rs1799724	Caucasian	1	1.07 (0.78, 1.48)	0.41	0.680	NA	NA	F	NA	NA
	Asian	4	1.02 (0.92, 1.14)	0.46	0.650	0	0.460	F	0.734	0.783
	Overall	5	1.03 (0.93, 1.14)	0.56	0.570	0	0.610	F	1.000	0.786
Cytokines										
	Caucasian	6	0.26 (0.03, 0.49)	2.23	0.030	44	0.110	F	0.174	0.007
	Asian	3	1.26 (0.49, 2.03)	3.19	0.001	90	< 0.001	R	1.000	0.704
	SA	1	−0.19 (−0.46, 0.08)	1.40	0.160	NA	NA	F	NA	NA
	Serum	6	0.54 (0.08, 1.01)	2.29	0.020	89	< 0.001	R	0.138	0.036
	Plasma	2	0.65 (−1.0, 2.30)	0.77	0.440	98	< 0.001	R	NA	NA
	CSF	1	0.54 (−0.12, 1.20)	1.59	0.110	NA	NA	F	1.000	NA
	GSF	1	0.48 (−0.01, 0.97)	1.93	0.050	NA	NA	F	NA	NA
	Overall	10	0.56 (0.17, 0.94)	2.83	0.005	91	< 0.001	R	0.451	0.024

CI, confidence interval; F, fixed-effect model; M, model; N, number of studies; NA, not available; OR, odds ratio; R, random-effects model; SMD, standardized mean difference; SNP, single nucleotide polymorphism; SA, South America; PB, P value for Begg's test; PE, value for Egger's test.

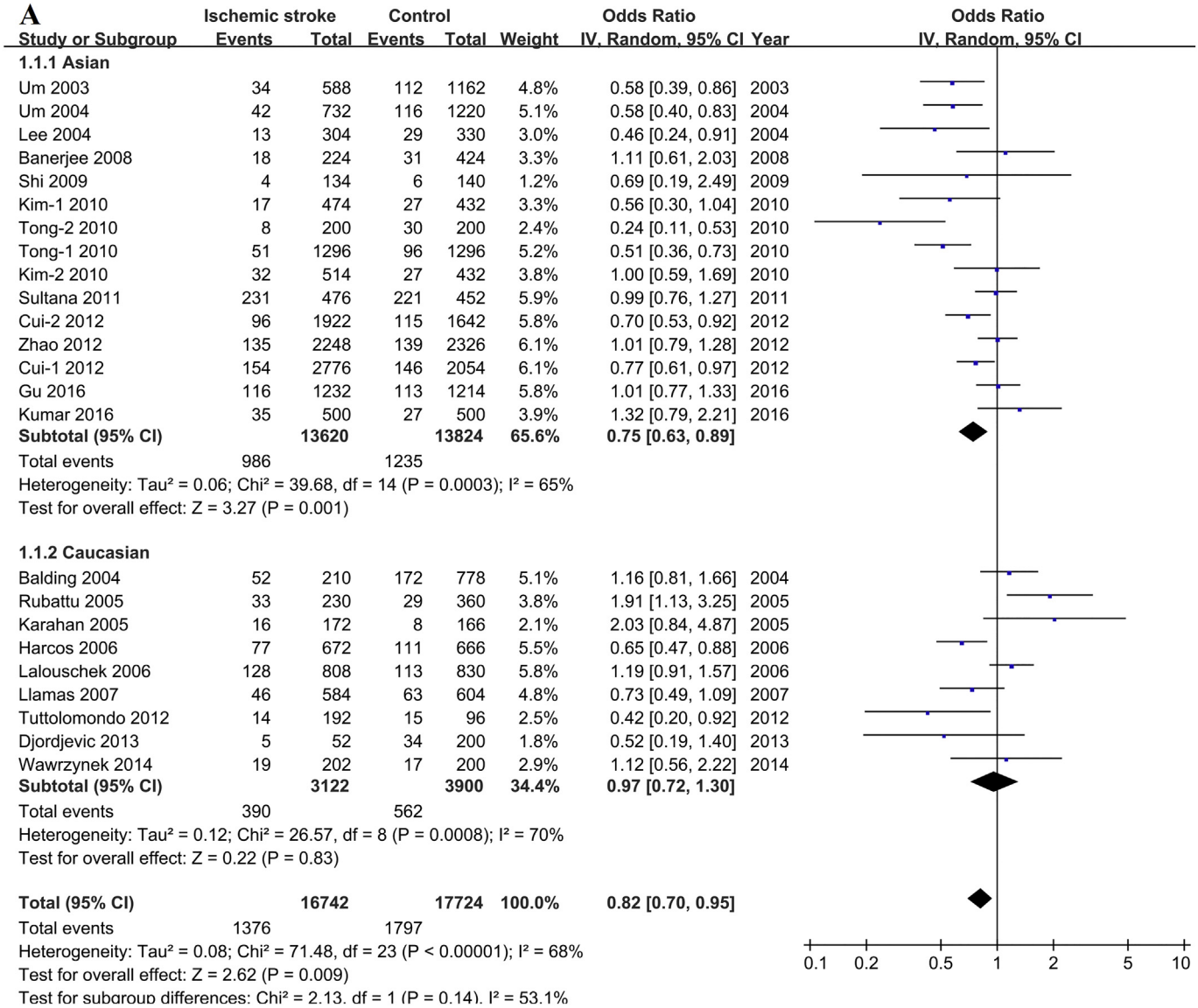

Fig. 2. Results of meta-analysis for the polymorphisms of rs1800629 (A), rs1800610 (B) and rs1799964 (C) and their forest plots.

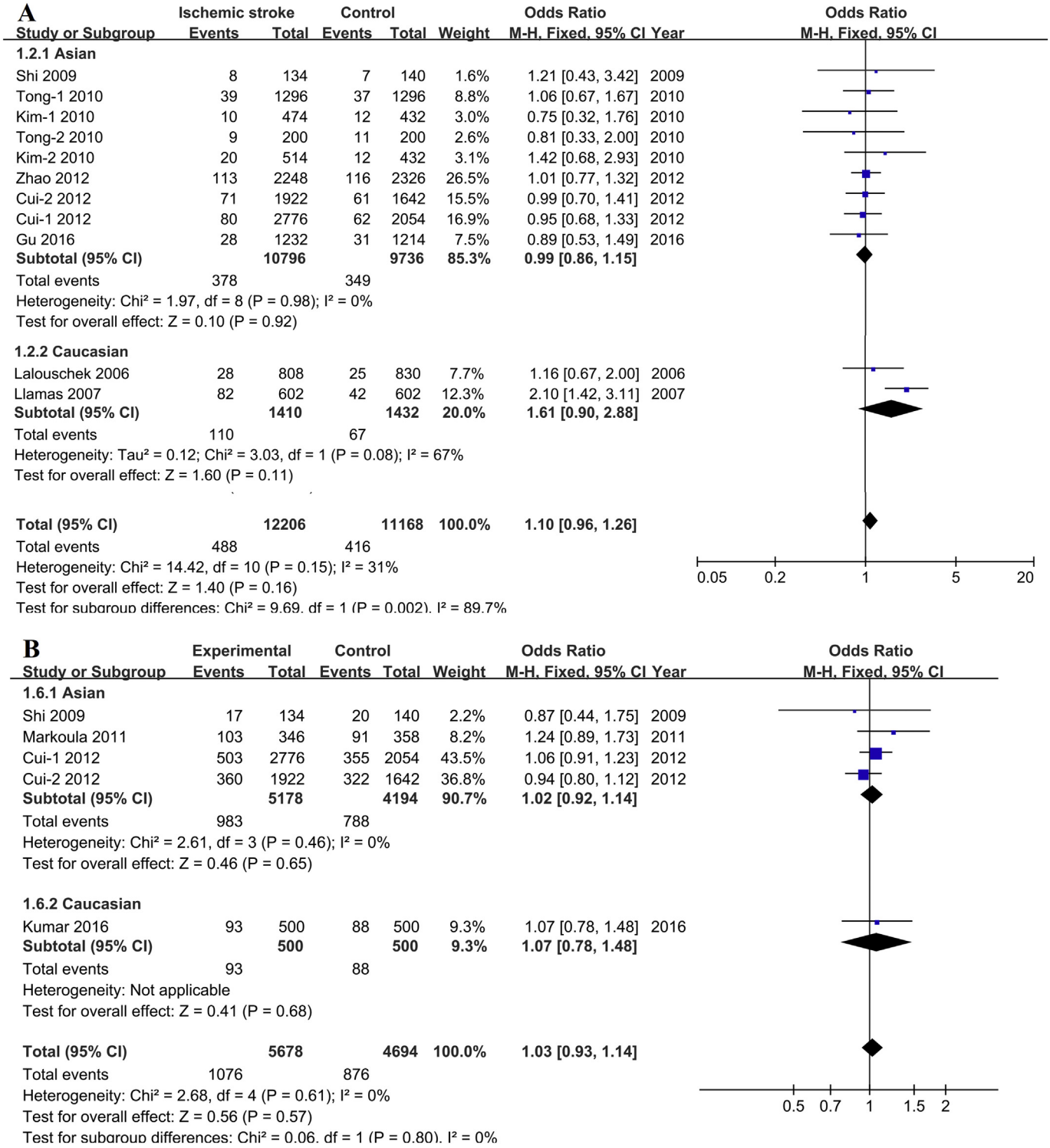

Fig. 3. Results of meta-analysis for the polymorphisms of rs361525 (A) and rs1799724 (B) and their forest plots.

analysis [47], we excluded an original article on investigating the relationship between rs1800629 and IS [48]; this may lead to confounding bias due to that IS patients were suffered from other cerebrovascular diseases. These results suggest that rigorous inclusion criteria and reasonable subgroup assignments can yield interesting results. Similar to previous studies [8,26,47], we failed to discover the role of rs361525 polymorphism in the susceptibility of IS. However, Niu et al. [49] suggested that the rs361525 polymorphism was a risk factor for IS in Asian adults, but did not support the role of rs1800629 in IS patients. Their meta-analysis is different from ours, probably because we only included articles published in the English language while they included articles in other languages. To date, a study has shown that rs361525 polymorphism was a genetic risk factor for IS patients in the Spanish [9], others did not find [8,21,23,25,27]. We hypothesized that rs361525 may not be a risk variation in the development and progression of IS, but this guess needs further validation. Compared with previous meta-analyses [45,47], our study first explored the association between rs1799964 polymorphism and IS susceptibility. In line with

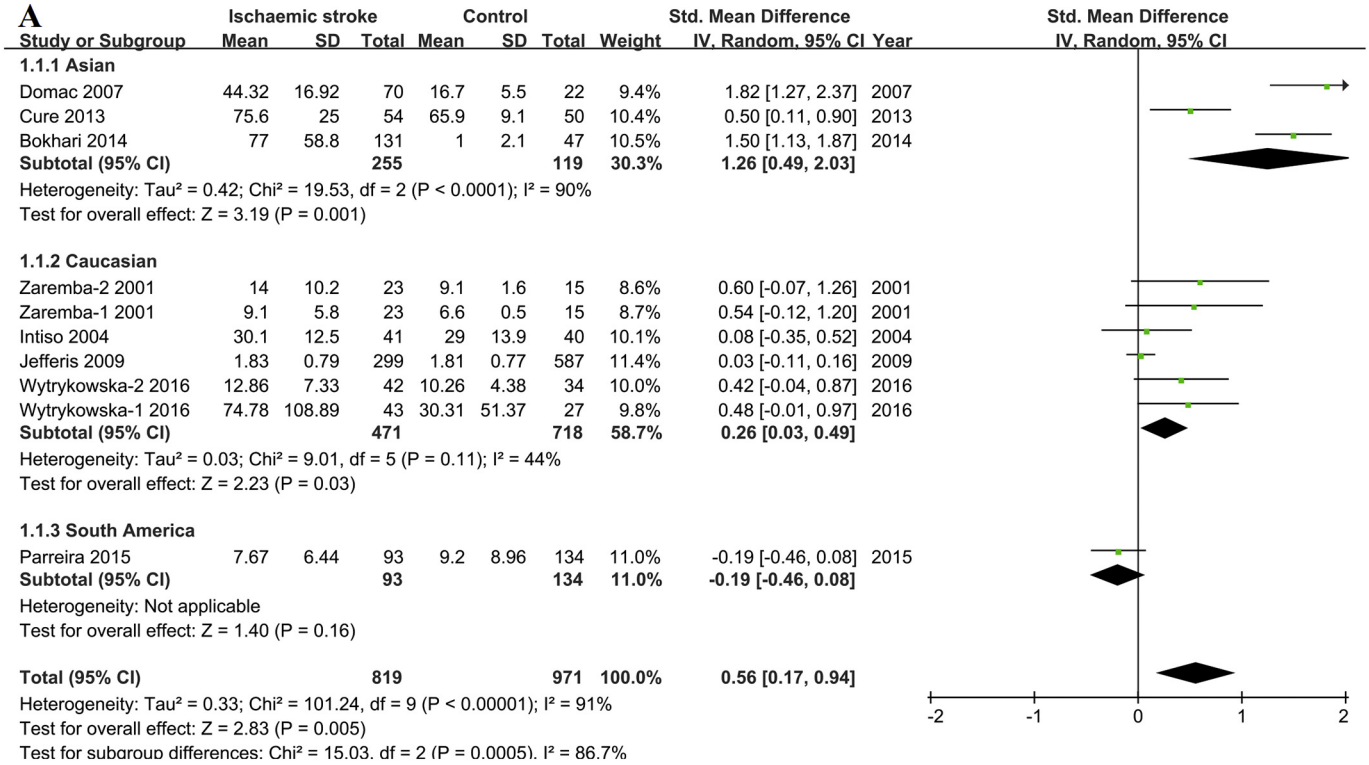

Fig. 4. Results of meta-analysis for the level of TNF-α cytokine (A represents stratified for ethnicity and B for specimen source).

previous studies [10,23], our results did not show an association between rs1799964 variation and IS risk. However, Cui et al. [8] found that the rs1799964 was closely related to the increased risk of IS in their first phase study, but it was not replicated in the second phase. Therefore, further researches are needed to determine whether the rs1799964 mutation is related to IS. Interesting, these original studies were conducted in the Asian populations, so we expected that future investigations will explore the relationship between rs1799964 polymorphism and IS susceptibility in Caucasians. In addition, there is a strong linkage disequilibrium between rs1799964 polymorphism and rs1800629 (D' = 0.74) [50], suggesting that the rs1799964 and its interaction with rs1800629 may have an important role in IS.

More importantly, we first systematically evaluated the association between TNF-α circulating level and IS risk by searching all relevant studies. Our results suggested that the TNF-α cytokine level was elevated in Asian, Caucasian and overall patients. Contrary to our meta-analysis, previous studies found no significant difference in the TNF-α level between Caucasian IS patients and controls [14,17,40,43]. One explanation is that the sample size in these original studies is smaller than ours, our findings are more convincing. Intriguingly, Parreira et al. [44] showed that the level of TNF-α in the controls was higher than that in Brazilian IS patients, but there was no significantly statistical difference. The difference in concentration of TNF-α cytokine between Asians and Caucasians may be due to genetic factors. TNF-α cytokine secretion was partly determined by hereditary factors [51], and polymorphisms of the TNF-α gene promoter have been associated with altered TNF-α expression and elevated TNF-α level [8,52,53]. For example, rs361525 genotype was related to a high cytokine phenotype [54], and the promoter region of rs1800629 may affect transcription of the TNF-α gene [55]. Furthermore, TNF-α increases the expression of adhesion molecules in endothelial cells of the central nervous microvascular system [56] and contributes to the recruitment and activation of leukocytes, and interaction of leukocyte and endothelium, further leading to proliferation of the smooth muscle cells and thickening of the arterial wall, and conversion of endothelium to a prothrombotic state [57]. In addition, numerous studies have explored the functional significance of rs1800629 polymorphism. Allele A of the rs1800629 was strongly associated with the MHC haplotype HLA-A1, B8, DR3 [55,58], which was associated with high TNF-α level [59,60]. Some studies have shown that the rs1800629 polymorphism affects transcription factor binding and further influences TNF-α transcription and expression levels, with the region in the A allele showing altered binding characteristics [60]. Therefore, we suspected that the variations of the TNF-α gene are related to the IS susceptibility by altering the level of TNF-α cytokine. In addition, the polymorphism of rs1800629 has been associated with the increased mRNA expression of TNF-α during the transcription process [55]. The median mRNA level of TNF-α in patients with acute IS was significantly higher than that in healthy controls [61,62]. Therefore, further studies should explore the role of genome (SNP)-transcriptome (mRNA)-cytokine axis in the inflammatory process of IS, which will help us to improve our understanding of the pathogenesis of IS. In the stratification analysis of specimen sources, an elevated level of TNF-α was found in serum but not in others, suggesting that the disease progression may be accompanied by changes in the blood [63]. Moreover, peripheral blood is more easily available than cerebrospinal fluid, so we can use blood specimens to explore the association between TNF-α and IS in future studies.

Sensitivity analysis and publication bias indicated that our results are stable. However, some limitations should be considered. First, records written in other languages were excluded, which may bias the true findings. Second, the sample size for studies on IS and TNF-α level was limited, so studies with a larger sample size should be expected. Third, studies on associations of rs361525 and rs1799964 polymorphisms with IS susceptibility were mainly performed in Asians, thus researches are needed in different ethnicities. Finally, longitudinal and experimental studies are required to validate whether the elevated the TNF-α levels and gene polymorphisms are the pathogenesis of stroke.

In conclusion, out current meta-analysis suggests that rs1800629 variant (G → A) can be considered as a protective factor for IS (especially in Asians) and rs1800610 (G → A) is a risk factor for Asian IS patients, and the circulating levels of TNF-α are elevated in Caucasian and Asian patients. Therefore, TNF-α plays an important role in the pathogenesis and process of IS and is a promising therapeutic target for the treatment of IS.

Abbreviations

CTR	Control
CSF	Cerebrospinal fluid
CI	Confidence interval
F	Fixed-effect model
GSF	Gingival crevicular fluid
IS	Ischemic stroke
M	median
M	Model
N	Number of studies
NA	Not available
NOS	Newcastle–Ottawa quality assessment scale
OR	Odds ratio
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PB	*P* value for Begg's test
PE	P value for Egger's test
R	Random-effects model
SMD	Standardized mean difference
SA	South America
SNP	Single nucleotide polymorphism
TNF-α	Tumor necrosis factor

Ethics approval and consent to participate

All analyses have been based on publicly available summary statistics and not individual data, so neither ethical approval from an institutional review board nor informed patient consent was required.

Consent for publication

All authors gave their consent for publication.

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Availability of data and material

The datasets are available from the corresponding author on reasonable request.

Declaration of competing interest

All authors declare they have no conflicts of interest.

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Authorship

Conception and design of the study: J-C W and S Q. Perform

research: J-C W, X Z and J-H W. Draft the article: J-C W and S Q. Analyze data: X Z. Acquisition of data: X Z, Q-W L, X-Q W, Z-Q W, J W, C Z.

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