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Association between the interleukin-6 -174 G/C polymorphism and risk of ischemic stroke: a meta-analysis

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ABSTRACT. Numerous studies have evaluated the association between the -174 G/C polymorphism in the interleukin-6 (*IL6*) gene and ischemic stroke risk. However, the results have been inconsistent. In this study, we performed a meta-analysis to assess the association of the *IL6* -174 G/C polymorphism with ischemic stroke. Published literatures from PubMed and Embase databases were retrieved. Pooled ORs with 95%CIs were calculated using fixed- or random-effect models. A total of seven case-control studies containing 2025 patients and 2174 controls were enrolled into this meta-analysis. In combined analysis, the results showed no significant association between the *IL6* -174 G/C polymorphism and ischemic stroke risk in the overall population (GG *vs* CC: OR = 1.22, 95%CI = 0.50-3.01; TT *vs* TC: OR = 0.97, 95%CI = 0.81-1.15; dominant: OR = 0.98, 95%CI = 0.70-1.38; or recessive: OR = 1.24, 95%CI = 0.57-2.70) models. In the subgroup analysis by race, no significant associations between the -174 G/C

polymorphism in the *IL6* gene and ischemic stroke risk were found in Caucasians or Asians. No publication bias was found in the present study (all $P > 0.05$). Overall, the meta-analysis results suggested that the *IL6* -174 G/C polymorphism was not associated with an increased risk of ischemic stroke. Further large and well-designed studies are needed to confirm this conclusion.

Key words: IL-6; -174 G/C; Gene polymorphism; Ischemic stroke

INTRODUCTION

Stroke is recognized as one of the leading causes of death and severe neurological disability worldwide (Lopez et al., 2006). The World Health Organization (WHO) has estimated that stroke affects 15 million people worldwide. Five million of these patients suffer from permanent disability and approximately 5.5 million others died from stroke-related factors (Banerjee et al., 2008). Ischemic stroke is the most common type of stroke, accounting for roughly 83% of all strokes (Rothwell et al., 2005). Traditional factors such as hypertension and smoking account for a significant proportion of the risk for ischemic stroke, but the factors contributing to much of the risk remain unknown (Meschia et al., 2011). However, with the recent developments in molecular biology, researchers have provided strong evidence that genetic factors play important roles in the pathogenesis of ischemic stroke (Flossmann et al., 2004).

The lesions of atherosclerosis represent the consequences of a series of highly specific cellular and molecular responses that can be best described as an inflammatory disease (Ross, 1999). Such inflammation-related atherosclerosis plays a crucial role in the progress and prognosis of ischemic stroke. Interleukin-6 (IL-6) is one of the most potent pro-inflammatory cytokines released during acute inflammation, which induces and regulates the production of acute phase proteins (Lehrer et al., 2005). *IL6* has been implicated in increasing the risk of stroke, and the expression level of serum IL-6 has been found to be elevated following acute stroke (Kim et al., 1996).

The *IL6* gene, located on chromosome 7p21, is composed of five exons, four introns, and a proximal promoter region (Bowcock et al., 1988). Functional polymorphisms in the promoter region of *IL6* are associated with increased plasma levels of this cytokine, and the best characterized genetic variant of *IL6* is a G-to-C substitution at position -174 (-174 G/C, rs1800795) upstream of the transcription start site, which has been reported to influence IL-6 levels (Fishman et al., 1998). Previous studies have shown that the -174 G/C polymorphism was associated with several diseases including coronary artery disease and Alzheimer's disease (Sie et al., 2006; Dai et al., 2012).

To date, many studies had been performed to evaluate the relationship between the -174 G/C polymorphism in the *IL6* gene and ischemic stroke risk. However, the results remain controversial. Meta-analysis can be a useful tool in detecting an association that could otherwise remain masked in studies of limited sample size, especially in those evaluating rare allele frequency polymorphisms (Attia et al., 2003). In the present study, we investigated whether the *IL6* -174 G/C polymorphism was associated with ischemic stroke risk by performing a meta-analysis.

MATERIAL AND METHODS

Literature search

PubMed and Embase databases were searched separately by two reviewers to retrieve papers linking the *IL6* -174 G/C polymorphism and ischemic stroke risk available by November 2014 without language restrictions, using the following key words: "interleukin-6/IL6", "-174 G/C", "ischemic stroke", "polymorphism", "single nucleotide polymorphism", and "genetic polymorphism". Search results were restricted to human populations and articles written in the English language. The full texts of the retrieved articles and reviews were scrutinized to decide whether information on the topic of interest was included. The reference lists of the original studies and review articles were also checked to ensure that relevant articles that were not initially identified were included in the meta-analysis. If more than one geographically or ethnically heterogeneous group was reported in any article, each group was treated separately.

Study selection

Citations selected from this initial search were screened for eligibility using the follow criteria: 1) case-control studies that addressed patients with ischemic stroke and healthy controls; 2) studies on the association of the *IL6* -174 G/C polymorphism and susceptibility to ischemic stroke; 3) studies that included sufficient genotype data for extraction; and 4) healthy controls were in Hardy-Weinberg equilibrium (HWE). The following were excluded: 1) studies not of case-control design that evaluated the association between the *IL6* -174 G/C polymorphism and ischemic stroke risk; 2) case reports, letters, reviews, meta-analyses, and editorial articles; 3) reports in which the number of null- and wild-type genotypes could not be ascertained; 4) studies that included duplicate data; and 5) healthy controls that were not in HWE.

Data extraction

Two reviewers abstracted the data independently and reached consensus on all subjects. The following data were abstracted directly from the studies included: first author, year of publication, area, numbers of patients and controls, distributions of genotypes and alleles, and evidence of HWE; these are listed in Table 1.

Statistical analysis

The Fisher exact test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was used to test the distributions of genotypes among controls for conformance with HWE. For each study, the numbers of the three genotypes (CC, GC, GG) in the patient and control groups were used as pooled data. The strengths of the associations between the -174 G/C polymorphism in the *IL6* gene and the susceptibility to ischemic stroke were estimated by ORs and their 95%CIs under homozygote comparison (GG *vs* CC), heterozygote comparison (GG *vs* GC), and dominant (CC+GC *vs* GG), and recessive (GG+GC *vs* CC) model comparisons between groups. We

quantified the effect of heterogeneity using the I^2 test. I^2 ranges between 0 and 100% and represents the proportion of inter-study variability that can be attributed to heterogeneity rather than to chance. I^2 values of 25, 50, and 75% were defined as low, moderate, and high estimates, respectively. When $I^2 > 50\%$ indicated heterogeneity across studies, the random-effect model was used for meta-analysis; else, the fixed-effect model was used. To evaluate ethnicity-specific effects, subgroup analyses were performed to explore and explain the diversity among the results of different studies. Sensitivity analysis was performed through by comparison of the random-effect model values with those of the fixed-effect model. Funnel plot asymmetry was assessed by the Begg test to estimate the potential publication bias ($P < 0.05$ was taken as representative of statistical significance). All statistical analyses were performed using STATA version 12.0 (Stata Corporation, College Station, TX, USA). All reported probabilities (P values) were two-sided, with P values <0.05 considered representative of statistical significance.

RESULTS

Study characteristics

The search strategy retrieved 21 potentially relevant studies. Base on the inclusion criteria, 7 case-control studies with full-text were included in this meta-analysis (Revilla et al., 2002; Balding et al., 2004; Flex et al., 2004; Chamorro et al., 2005; Lalouschek et al., 2006; Banerjee et al., 2008; Tong et al., 2010) and 14 studies were excluded. The flow chart for the study selection is summarized in Figure 1. These 7 case-control studies selected included a total of 2025 patients and 2174 healthy controls. The HWE test was performed on the genotype distributions of the controls; all were in HWE ($P > 0.05$). Of the 7 studies included, 5 were of Europeans (Revilla et al., 2002; Balding et al., 2004; Flex et al., 2004; Chamorro et al., 2005; Lalouschek et al., 2006) and 2 were of Asians (Banerjee et al., 2008; Tong et al., 2010). The publishing year of the studies included ranged from 2000 to 2014. The general characteristics and the allele and genotype distributions in the published articles included in this meta-analysis are shown in Table 1.

Quantitative synthesis

A summary of the meta-analysis findings of the association between the *IL6* -174 G/C polymorphism and ischemic stroke risk is shown in Table 2 and in Figure 2. We found no significant association between the *IL6* -174 G/C polymorphism and ischemic stroke risk in any genetic model tested (GG *vs* CC: OR = 1.22, 95%CI = 0.50-3.01; GG *vs* GC: OR = 0.97, 95%CI = 0.81-1.15; dominant model: OR = 0.98, 95%CI = 0.70-1.38; or recessive model: OR = 1.24, 95%CI = 0.57-2.70). When subjects were stratified according to ethnicity, no significant association was detected in Caucasians (GG *vs* CC: OR = 1.08, 95%CI = 0.43-2.75; GG *vs* GC: OR = 0.99, 95%CI = 0.82-1.20; dominant model: OR = 0.99, 95%CI = 0.66-1.49; or recessive model: OR = 1.12, 95%CI = 0.50-2.49) or Asians (GG *vs* CC: OR = 7.10, 95%CI = 0.38-133.17; GG *vs* GC: OR = 0.87, 95%CI = 0.57-1.34; dominant model: OR = 1.08, 95%CI = 0.70-1.65; or recessive model: OR = 7.62, 95%CI = 0.41-142.48). Sensitivity analyses were conducted by altering the statistic models. No material alterations in the results were detected, indicating that our results were statistically robust.

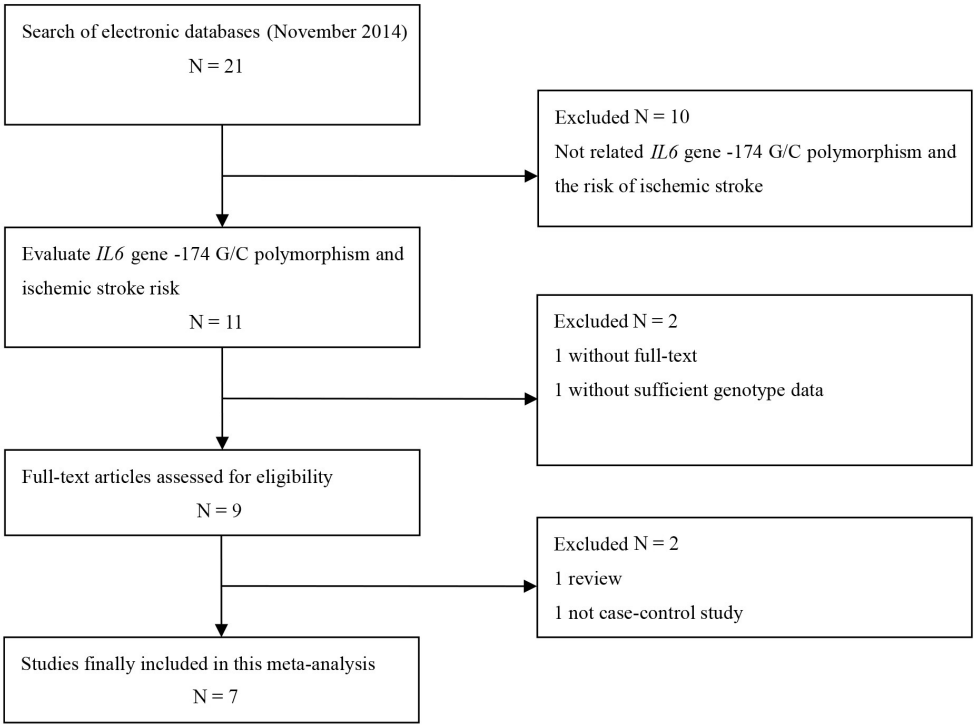

Figure 1. Flow diagram of study searching and selection process.

Table 1. Characteristics of the studies included for meta-analysis.

Studies included	Area	Ethnicity	Cases/Controls	Genotypes for cases			Genotypes for controls			HWE test
				CC	GC	GG	CC	GC	GG	
Revilla et al. (2002)	Spain	Caucasian	82/82	15	40	27	6	39	37	0.32
Balding et al. (2004)	Ireland	Caucasian	105/389	12	60	33	68	198	123	0.44
Flex et al. (2004)	Italy	Caucasian	237/223	22	115	100	68	99	56	0.10
Chamorro et al. (2005)	Spain	Caucasian	273/105	35	134	104	9	50	46	0.37
Lalouschek et al. (2006)	Austria	Caucasian	404/415	74	187	143	67	192	156	0.54
Banerjee et al. (2008)	India	Asian	176/212	0	53	123	4	52	156	0.89
Tong et al. (2010)	China	Asian	648/648	0	0	648	0	3	645	0.95
Tong et al. (2010)	China	Asian	100/100	0	1	99	0	2	98	0.92

Table 2. Meta-analysis for association of the *IL6* -174 G/C polymorphism with ischemic stroke.

Variables	N	Cases/Controls	GG *vs* CC	GG *vs* GC	Dominant model	Recessive model
			OR (95%CI) P I^2	OR (95%CI) P I^2	OR (95%CI) P I^2	OR (95%CI) P I^2
Total	7	2025/2174	1.22 (0.50-3.01) 0.00 87.9%	0.97 (0.81-1.15) 0.26 21.6%	0.98 (0.70-1.38) 0.00 67.1%	1.24 (0.57-2.70) 0.00 86.4%
Ethnicity						
Caucasian	5	1101/1214	1.08 (0.43-2.75) 0.00 90.0%	0.99 (0.82-1.20) 0.21 31.6%	0.99 (0.66-1.49) 0.00 78.4%	1.12 (0.50-2.49) 0.00 88.6%
Asian	2	924/960	7.10 (0.38-133.17) -	0.87 (0.57-1.34) 0.27 24.2%	1.08 (070-1.65) -	7.62 (0.41-142.48) -

N = number; I^2 = inconsistency index; CI = confidence interval; OR = odds ratio.

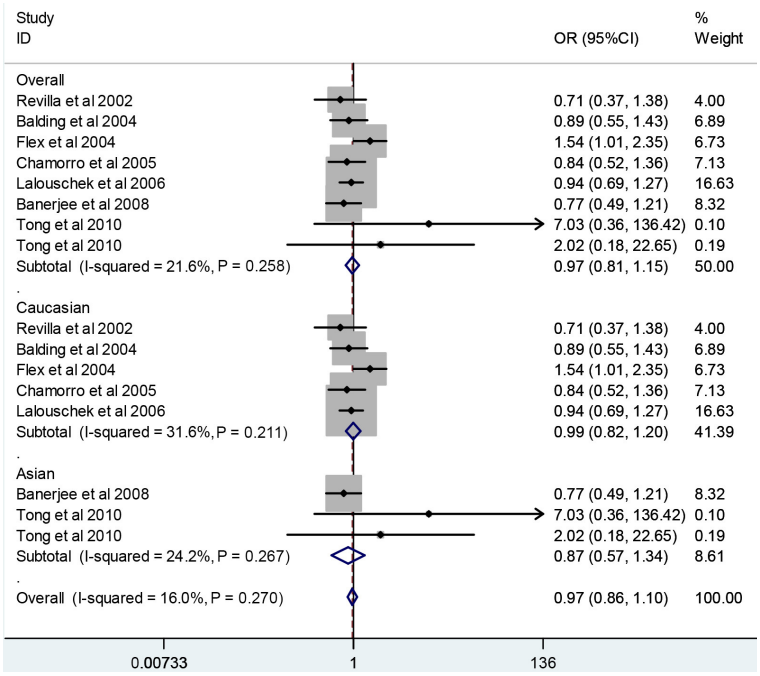

Figure 2. Meta-analysis of the relationship between the -174 G/C polymorphism in the *IL6* gene and ischemic stroke risk (GG *vs* GC).

Publication bias

A funnel plot was used to assess the publication bias. There was no evidence of publication bias visually from the funnel plot (Figure 3), which implied that the publication bias was low in the present meta-analysis (all $P > 0.05$).

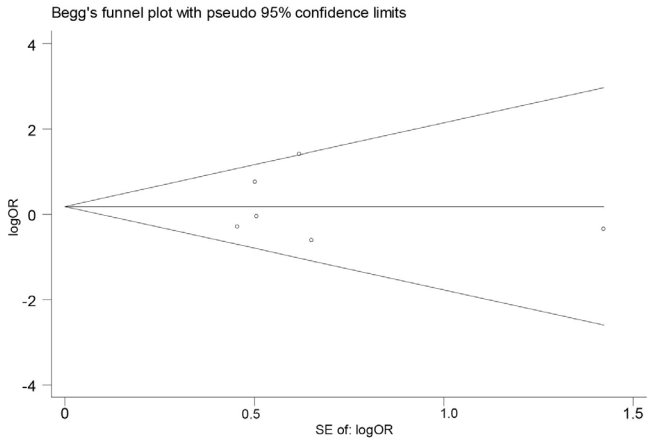

Figure 3. Begg funnel plot test of publication bias for association of the -174 G/C polymorphism in the *IL6* gene with ischemic stroke risk (GG *vs* GC).

DISCUSSION

Ischemic stroke is a multifactorial disease and its pathogenesis is not yet fully understood. It is now accepted that both genetic and environmental factors contribute to ischemic stroke susceptibility and outcome. Similarly, the inflammatory reaction is also relevant to ischemic stroke (Ross, 1999). IL-6 is one of the confirmed major pleiotropic pro-inflammatory cytokines associated with cardiovascular diseases including stroke, and elevated serum IL-6 has been found in patients with acute ischemic stroke (Ridker et al., 2000). Recently, a variety of studies have focused on the association between the *IL6* -174 G/C polymorphism and ischemic stroke; however, the results of these studies are controversial. The most likely reason for the inconsistencies among these studies is that they are single case-control studies with small sample sizes. Meta-analysis is a powerful method to enhance the statistical power of the analysis through increasing the sample size from that of individual studies. Here, we conducted a meta-analysis to explore the association between the -174 G/C polymorphism in the *IL6* gene and ischemic stroke risk.

This is the first systematic study of the association between the *IL6* -174 G/C polymorphism and ischemic stroke risk using meta-analysis. Following literature search and screening, 7 case-control studies were included and assessed, including a total of 2025 patients with ischemic stroke and 2174 healthy controls. The results suggested that there was no significant association between the *IL6* -174 G/C polymorphism and ischemic stroke risk in the overall population. Considering that the result might be affected by ethnicity, we performed a race-related subgroup analysis, which revealed that the *IL6* -174 G/C polymorphism was not associated with an increased or decreased risk of ischemic stroke in either Caucasians or Asians. Sensitivity analysis was performed by the comparison of random- and fixed-effect model values, and the result revealed that this meta-analysis was realistic and believable. Nevertheless, caution should be exercised when considering our conclusion. No evidence was found to indicate publication bias in this meta-analysis.

The functional effect of the *IL6* -174 G/C polymorphism might have limited impact on ischemic stroke. As with other diseases, the development of ischemic stroke is due to the joint effects of multiple genes and gene-environment interactions. Evidence suggests that the -597G/A, -572G/C, -373A(n)T(n), and -174G/C polymorphisms in the *IL6* gene synergistically increase the risk of ischemic stroke (Acalovschi et al., 2003). In addition, gene-environment interactions should be taken into consideration in future analysis, which should lead to a better, more comprehensive understanding of the association between the *IL6* -174 G/C polymorphism and ischemic stroke risk.

The present study has some limitations. First, our meta-analysis was based on unadjusted OR estimates because not all published studies presented adjusted ORs, or when they did, the ORs were not adjusted by the same potential confounders such as age, gender, and exposures. Lacking information for data analysis might cause serious confounding bias. Second, the number of studies and the numbers of subjects in the studies included in the meta-analysis by specific subgroups were small. Finally, only published English studies were included in this study; therefore, publication and potential language biases might occur.

In conclusion, this meta-analysis suggested that the -174 G/C polymorphism in the *IL6* gene might be not associated with ischemic stroke risk. Further studies estimating the effect of gene-gene and gene-environment interactions might eventually provide a better, more comprehensive understanding of this association.

Conflicts of interest

The authors declare no conflict of interest.

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