



# Interleukin-18 gene promoter 607A polymorphism, but not 137C polymorphism, is a protective factor for ischemic stroke in the Chinese population: A meta-analysis



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## ABSTRACT

Some epidemiological studies have evaluated the association between interleukin (IL)-18 promoter polymorphisms and the risk of ischemic stroke (IS), but the results were inconsistent. The present meta-analysis was therefore performed to investigate the relationship between IL-18 promoter 137G/C and 607C/A polymorphisms and the risk of IS in the Chinese population. Related studies from PubMed, Embase, Web of Science, CBMdisc and CNKI databases up to November 1, 2014 were systematically searched, also the reference lists of identified articles were manually searched. Information was extracted to calculate for the allelic, genotypic, dominant and recessive models using the pooled odds ratios (ORs) along with 95% confidence intervals (CIs). Evidence of significant association between 607C/A polymorphism and risk of IS was found in four genetic models based on the overall population. However, no significant association between 137G/C polymorphism and risk of IS was found in four genetic models. In summary, the present study suggests that IL-18 gene promoter 607A polymorphism is a protective factor for IS in the Chinese population, while 137C polymorphism has weaker or no protective properties. Still, a larger number of studies with large scale and sufficient original information are required to further confirm our findings.

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## 1. Introduction

China has approximately 2.5 million new strokes per year and stroke has become the leading cause of death and adult disability in the country (Liu et al., 2011). Ischemic stroke (IS) is the most common type of stroke in China and accounts for 43% to 79% of all stroke events (Liu et al., 2011). The precise pathophysiology of IS remains unclear. Besides the conventional stroke risk factors, accumulating evidence suggests that genetic differences play a crucial role in the susceptibility of IS (Sharma et al., 2013; Markus and Bevan, 2014). Recent studies using innovative technologies and methods further broaden our understanding of the role of genetic variations in stroke (Falcone et al., 2014). Many genes have been associated with IS susceptibility, hence these genes have been proposed as novel promising targets for stroke prevention and treatment (Sharma et al., 2013; Markus and Bevan, 2014).

The cytokine interleukin-18 (IL-18) was initially identified as a protein that induces interferon  $\gamma$  (IFN $\gamma$ ) production (Nakamura et al., 1993). IL-18 is a member of the IL-1 family of cytokines and was firstly

synthesized as an inactive precursor, which becomes active upon caspase-1-mediated cleavage (Novick et al., 2013). A variety of cells including Kupffer cells, monocytes, macrophages and dendritic cells can produce IL-18. IL-18 is capable of modulating the immune response via stimulating cytokine gene expression, promoting T helper cell differentiation and natural killer cell activation, and serving as a major pro-inflammatory cytokine in inflammatory and autoimmune diseases (Sedimbi et al., 2013). In the central nervous system, microglia, astrocytes and neurons can express receptors for IL-18 and thereby participate in local inflammation associated with many neurological diseases including meningitis, stroke and Alzheimer's disease (Walsh et al., 2014).

The IL-18 gene is located on q22.2-q22.3 locus of human chromosome 11 (Nolan et al., 1998). Two functional polymorphisms in the IL-18 promoter, i.e., G to C transition at position-137 (137G/C, rs187238) and C to A transition at position-607 (607C/A, rs1946518), could influence the binding of transcription factor and thus modify IL-18 mRNA expression. The 137G/C polymorphism changes the H4TF-1 nuclear factor binding site, while the 607C/A polymorphism disrupts the bind of potential cAMP-responsive element binding protein (Giedraitis et al., 2001). In addition, 137G/C polymorphism could reduce IL-18 transcriptional activity significantly (Liang et al., 2005). These two polymorphisms have been associated with various diseases including

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type 1 diabetes (Lee et al., 2015), chronic hepatitis C virus infections (Yang and Liu, 2015) and cancer (Li et al., 2015). Recently, conflicting results have been reported by some epidemiological studies from China (Chen et al., 2010; Zhang et al., 2010; Li et al., 2011; Wang and Cheng, 2011; Ren et al., 2012; Lu et al., 2013; Wang et al., 2013; Wei et al., 2013), which have focused on the relationship between IL-18 gene promoter polymorphisms (137G/C and 607C/A) and the risk of IS. By applying advanced systematic and quantitative approaches to results from multiple populational studies, meta-analysis can serve as a powerful tool for precise evaluation of clinical data and identification of emerging trends (Sagoo et al., 2009). The present meta-analysis was therefore designed to clarify the association between IL-18 gene promoter 137G/C and 607C/A polymorphisms and the risk of IS in the Chinese population.

## 2. Methods

### 2.1. Literature search

The present meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria (Moher et al., 2009). We searched relevant studies from PubMed, EMBASE, Web of Science, CBMdisc (Chinese Biomedical Literature on disc) and CNKI (China National Knowledge Infrastructure) up to November 1, 2014. The initial keywords for searching were used: (“interleukin-18” OR “IL-18”) AND (“polymorphism” OR “variant” OR “mutation” OR “genotype” OR “607C/A” OR “137G/C”) AND (“stroke” OR “ischemic stroke” OR “cerebral infarction” OR “cerebrovascular disease” OR “cerebrovascular disorder” OR “cerebral ischemia” OR “brain ischemia” OR “brain infarction”). In addition, the reference lists of retrieved articles were reviewed to find more studies. Studies with English and Chinese language were both included.

### 2.2. Inclusion criteria

Included studies met the following criteria: (1) Case-control studies on the association between IL-18 gene promoter polymorphisms (137G/C and/or 607C/A) and risk of IS in the Chinese population; (2) Diagnosis of IS was made by neurological examination and neuroimaging confirmation according to the revision of the diagnostic criteria in Chinese cerebrovascular disease conference; (3) Sufficient original data were required to estimate an odds ratio (OR) along with 95% confidence interval (CI); (4) Studies with full text articles; (5) The more recent or complete article was included when duplicate publications were present; (6) The cases were from the hospital and the controls were from healthy individuals who received physical examination during the same period. The control groups, matched for sex and age with the cases, were confirmed healthy and neurologically normal by medical history and general examinations. Reviews and studies without complete data were excluded. If needed, we can contact study authors to identify additional studies and to request missing data by phone or email.

### 2.3. Data extraction

Data were extracted independently by two authors (MJ Zhang and Y Zhou) in duplicate from all eligible studies. Agreements were reached after discussion for conflicting data, or the third author (LL Zhang) was consulted. The data for each study included name of first author, year of publication, source of controls, sample size and genotype distribution of cases and controls, calculation of the Hardy-Weinberg equilibrium (HWE) in control using Chi<sup>2</sup> test ( $P_H$  value < 0.05 was considered statistically significant, Program HWE version 1.20), mutant allele frequency in control and Newcastle Ottawa Scale (NOS) score.

### 2.4. Quality score assessment

The quality of each study was independently assessed by two authors (MJ Zhang and Y Zhou) using a numerical score ranging from 0 (worst) to 9 (best) according to Newcastle Ottawa Scale (NOS) (Wells et al., n.d.). Study with a score > 7 was considered as “high quality”. Disagreement was resolved by discussion or consultation (LL Zhang).

### 2.5. Statistical analysis

The pooled OR with 95%CI was used to measure the strength of association between IL-18 gene promoter polymorphisms and risk of IS by software of Review Manager 5.1 and STATA 11.0. ORs along with the corresponding 95%CIs were calculated for the allelic model (d allele vs. D allele, d was for the mutant allele and D was for the common allele), genotypic model (dd vs. DD, Dd vs. DD), dominant model (dd + Dd vs. DD) and recessive model (dd vs. Dd + DD), respectively. Heterogeneity was checked by the Cochran's Q test and I<sup>2</sup> statistic. The random-effects Mantel-Haenszel method was adopted if the result of the Q test was  $P_Q < 0.10$  or I<sup>2</sup> statistic was > 50%, which indicated the statistically significant heterogeneity between the studies. Otherwise, the fixed-effects Mantel-Haenszel method was used. To test the statistical power of this meta-analysis, power analysis was performed using the software “PS: Power and Sample Size Calculation” (Version 3.0.43). Statistical power ≥ 80% was a general threshold for acceptable analysis with low possibility of false negative. Subgroup analyses were conducted based on the subtypes of IS (LAA, large artery atherosclerosis; SVD, small vessel disease; CE, cardioembolism), age of subjects (younger than 65, abbreviated as ~65; and older than 65, abbreviated as 65~) or sex of subjects (male and female). Sensitivity analyses were performed by limiting the meta-analysis to studies with high quality (NOS score > 7) or studies meeting the HWE. The potential publication bias was assessed by Begg's funnel plots and Egger's regression test (Significance sets at  $P_E < 0.05$ ).

## 3. Results

### 3.1. Study characteristics

The present study met the PRISMA statements (Checklist S1). A total of seventy-three articles were identified after first searching. After comprehensive review and evaluation, eight articles involving twelve studies (Chen et al., 2010; Zhang et al., 2010; Li et al., 2011; Wang and Cheng, 2011; Ren et al., 2012; Lu et al., 2013; Wang et al., 2013; Wei et al., 2013) from China were included in the final meta-analysis through the selection process (Fig. 1). Of these, seven studies containing 1566 cases and 1372 controls investigated 137G/C polymorphism and five studies containing 1391 cases and 1194 controls investigated 607C/A polymorphism. All of twelve studies have population-based controls. Only one study included data on 607C/A polymorphism (Ren et al., 2012) didn't follow the HWE. The mean score of NOS was 7.92 with only one study provided data on 137G/C polymorphism (Wei et al., 2013) had a score < 8, indicating that the quality of included studies was generally good. These included studies and their main characteristics were present in Table 1.

### 3.2. Quantitative synthesis

No significant association between IL-18 gene promoter 137G/C polymorphism and risk of IS in Chinese population was found in all four genetic models (allelic model: OR = 0.91, 95%CI = 0.66–1.25,  $p = 0.55$ ; genotypic model: OR = 0.79, 95%CI = 0.50–1.27,  $p = 0.33$  (CC vs. GG); OR = 0.92, 95%CI = 0.64–1.32,  $p = 0.64$  (GC vs. GG); dominant model: OR = 0.91, 95%CI = 0.63–1.31,  $p = 0.61$ ; and recessive model: OR = 0.78, 95%CI = 0.49–1.24,  $p = 0.29$ , respectively, Fig. 2).

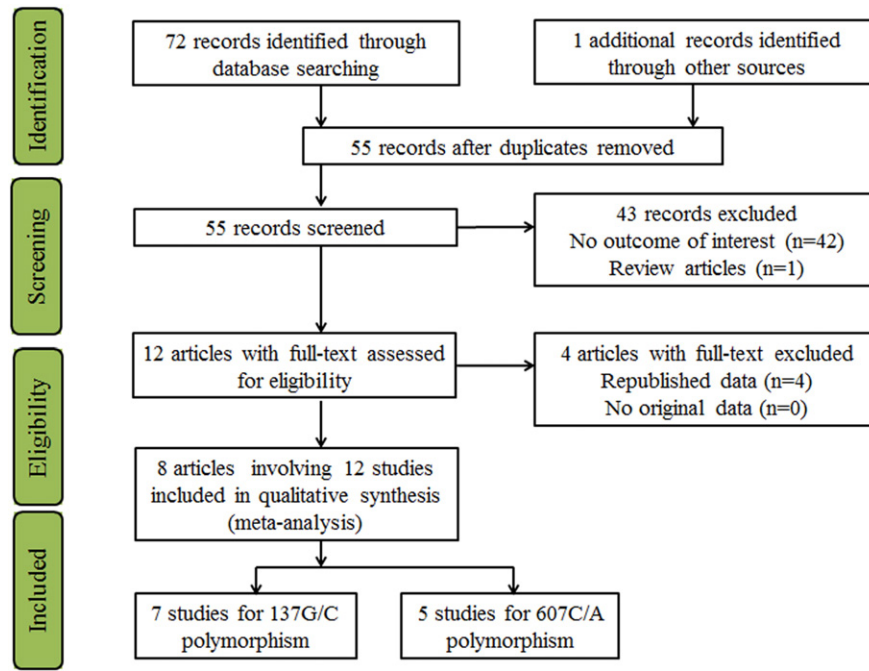


Fig. 1. A flow diagram of selection process to identify and retrieve eligible studies for inclusion.

Random-effects method was adopted in some genetic models because heterogeneity was observed ( $P_Q < 0.10$  or  $I^2 > 50\%$ ). In the subgroup analyses based on types of IS (LAA, SVD and CE), age of subjects (~65 and 65~) or sex of subjects (male and female), no significant association was found in most genetic models. However, we detected significant association with IS risk in allelic, genotypic (CC vs. GG), dominant and recessive models of LAA subgroup, in genotypic model (CC vs. GG) of 65 ~ subgroup, and in genotypic (CC vs. GG) and recessive models of male subgroup.

For the IL-18 gene promoter 607C/A polymorphism, a significant association with IS risk was found (allelic model: OR = 0.73, 95%CI = 0.65–0.81,  $p < 0.01$ ; genotypic model: OR = 0.51, 95%CI = 0.40–0.64,  $p < 0.01$  (CC vs. GG); OR = 0.68, 95%CI = 0.56–0.82,  $p < 0.01$  (GC vs. GG); dominant model: OR = 0.62, 95%CI = 0.52–0.74,  $p < 0.01$ ; and recessive model: OR = 0.67, 95%CI = 0.55–0.80,  $p < 0.01$ , respectively, Fig. 3). Fixed-effects method was adopted in all genetic models because no heterogeneity was observed ( $P_Q > 0.10$  or  $I^2 = 0$ ). In the subgroup analyses, significant association was also found in all genetic models of LAA subgroup, ~65 subgroup and male subgroup, as well as in genotypic (AA vs. CC) and recessive models of SVD subgroup.

Finally, power calculations on the overall sample size of 137G/C and 607C/A polymorphisms showed that the statistical powers were 0.248 and 1.000, respectively. We did not perform the subgroup analysis on source of controls, because the source of controls in all studies was population-based (not hospital-based). The main results of subgroup analyses were detailed in Table 2.

### 3.3. Sensitivity analysis

The sensitivity analysis was performed after limiting the included studies with NOS score > 7 or studies meeting the HWE. After limiting the studies with NOS score > 7 (137G/C) or studies meeting the HWE (607C/A), the corresponding pooled ORs didn't show any change (Table 2). These data suggest that our results were robust.

### 3.4. Publication bias

As we all know, published studies may not be truly representative of all valid studies undertaken, which is the source of publication bias. This bias may distort the results of a meta-analysis. In this article, Begg's

Table 1  
Main characteristics of studies included in this meta-analysis.

First author	Year	Source of controls	Sample size case/control	Genotypes distribution (case/control)			HWE Y/N(p)	MAF	NOS score	Stratification
				GG	GC	CC				
137G/C										
Chen	2010	PB	190/210	111/145	71/59	8/6	Y(1)	0.169	8	
Zhang	2010	PB	423/384	313/279	105/91	5/14	Y(0.062)	0.155	8	Subtypes of IS, Age, Sex
Li	2011	PB	98/100	76/62	19/33	3/5	Y(0.823)	0.215	8	
Wang	2011	PB	218/218	174/146	42/66	2/6	Y(0.652)	0.179	8	Subtypes of IS
Ren	2012	PB	193/120	161/96	29/23	3/1	Y(0.768)	0.104	8	
Wang	2013	PB	291/226	240/180	46/43	5/3	Y(0.813)	0.108	8	
Wei	2013	PB	153/114	91/85	54/25	8/4	Y(0.223)	0.145	7	
607C/A										
Zhang	2010	PB	423/384	122/81	227/207	74/96	Y(0.118)	0.480	8	Subtypes of IS, Age, Sex
Li	2011	PB	98/100	25/23	55/56	18/21	Y(0.228)	0.510	8	
Ren	2012	PB	193/120	58/17	99/71	36/32	N(0.027)	0.438	8	
Lu	2013	PB	386/364	116/77	188/195	82/92	Y(0.162)	0.479	8	Age, Sex
Wang	2013	PB	291/226	91/50	147/108	53/68	Y(0.566)	0.460	8	

HWE, Hardy-Weinberg equilibrium; Y, yes (pH value of HWE > 0.05); N, no (p value of HWE < 0.05); MAF, minor allele frequency in control; NOS, Newcastle Ottawa Scale; PB, population-based controls; IS, ischemic stroke.

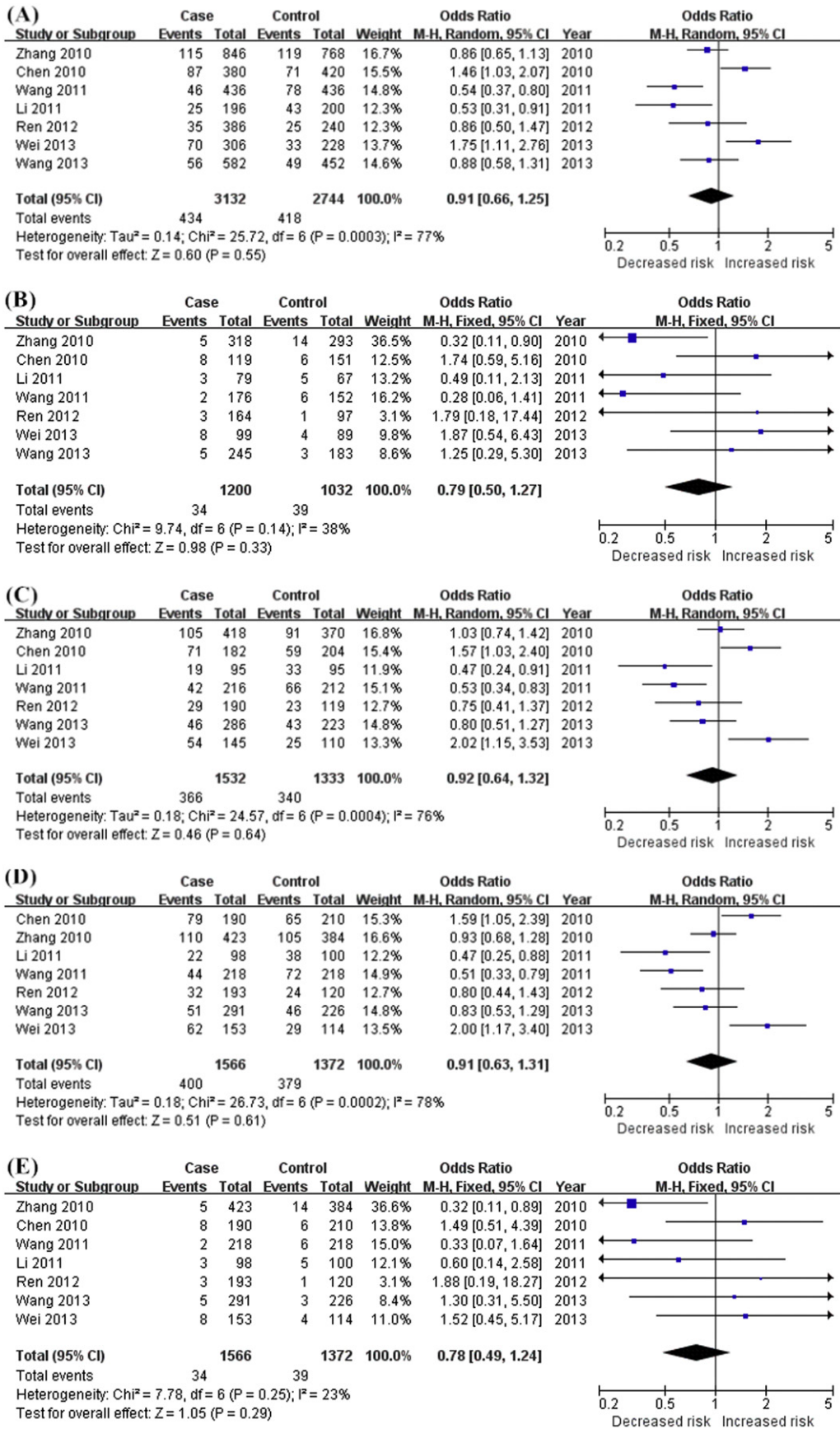
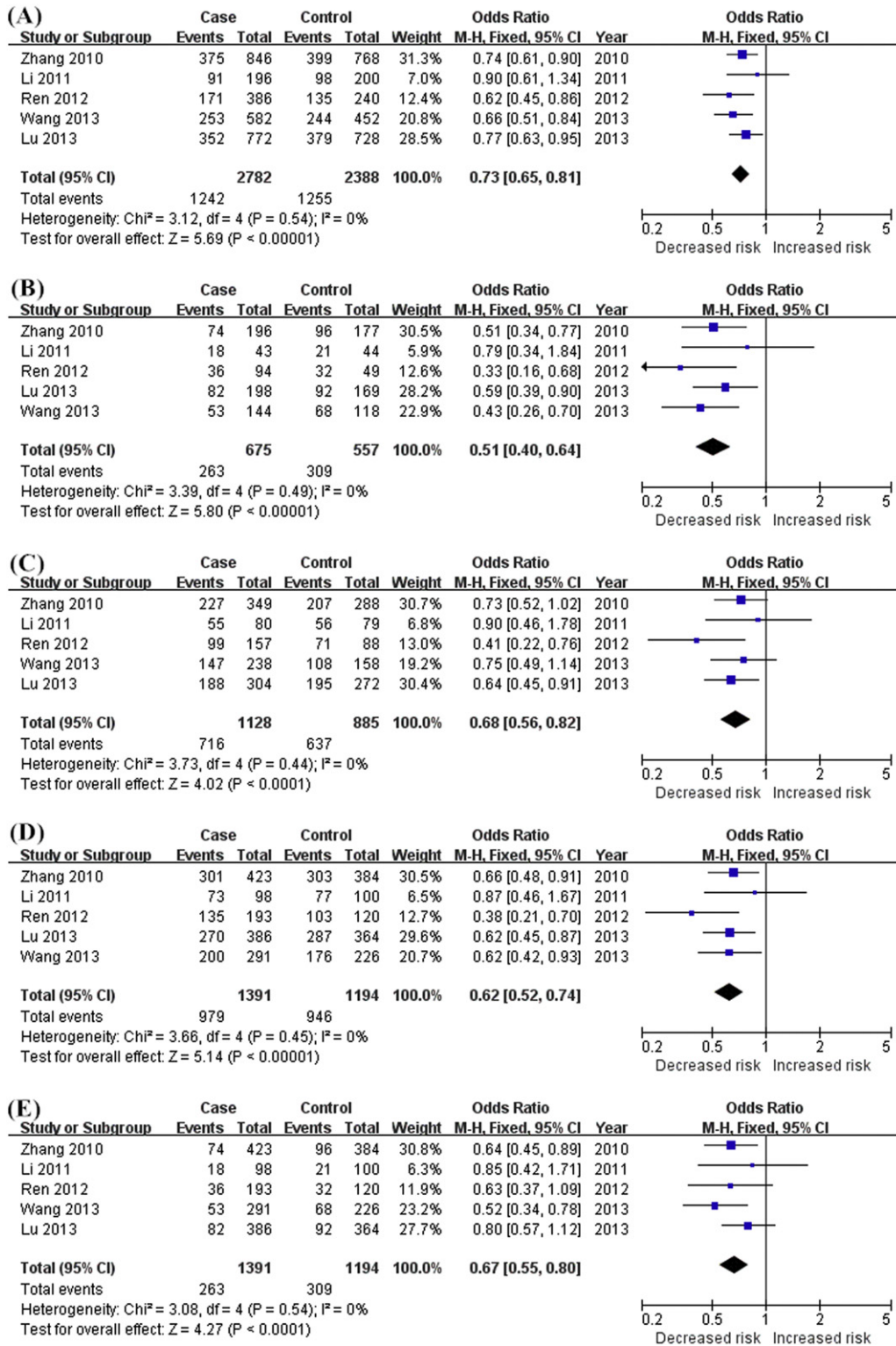


Fig. 2. Forest plots for IL-18 promoter 137G/C polymorphism and risk of ischemic stroke in the genetic models of C allele vs. G allele (A), CC vs. GG (B), GC vs. GG (C), CC + GC vs. GG (D), and CC vs. GC + GG (E).



**Fig. 3.** Forest plots for IL-18 promoter 607C/A polymorphism and risk of ischemic stroke in the genetic models of A allele vs. C allele (A), AA vs. CC (B), CA vs. CC (C), AA + CA vs. CC (D), and AA vs. CA + CC (E).

funnel plots (Begg and Mazumdar, 1994) and Egger's regression test (Egger et al., 1997) were performed to assess the potential publication bias. The funnel plot is based on the fact that precision in estimating the intervention factor effect will increase as the sample size increases. The skewed and asymmetrical funnel plots will be found in case of publication bias, and symmetrical funnel plots will be found otherwise. Egger's test is a linear regression approach to measure funnel plot

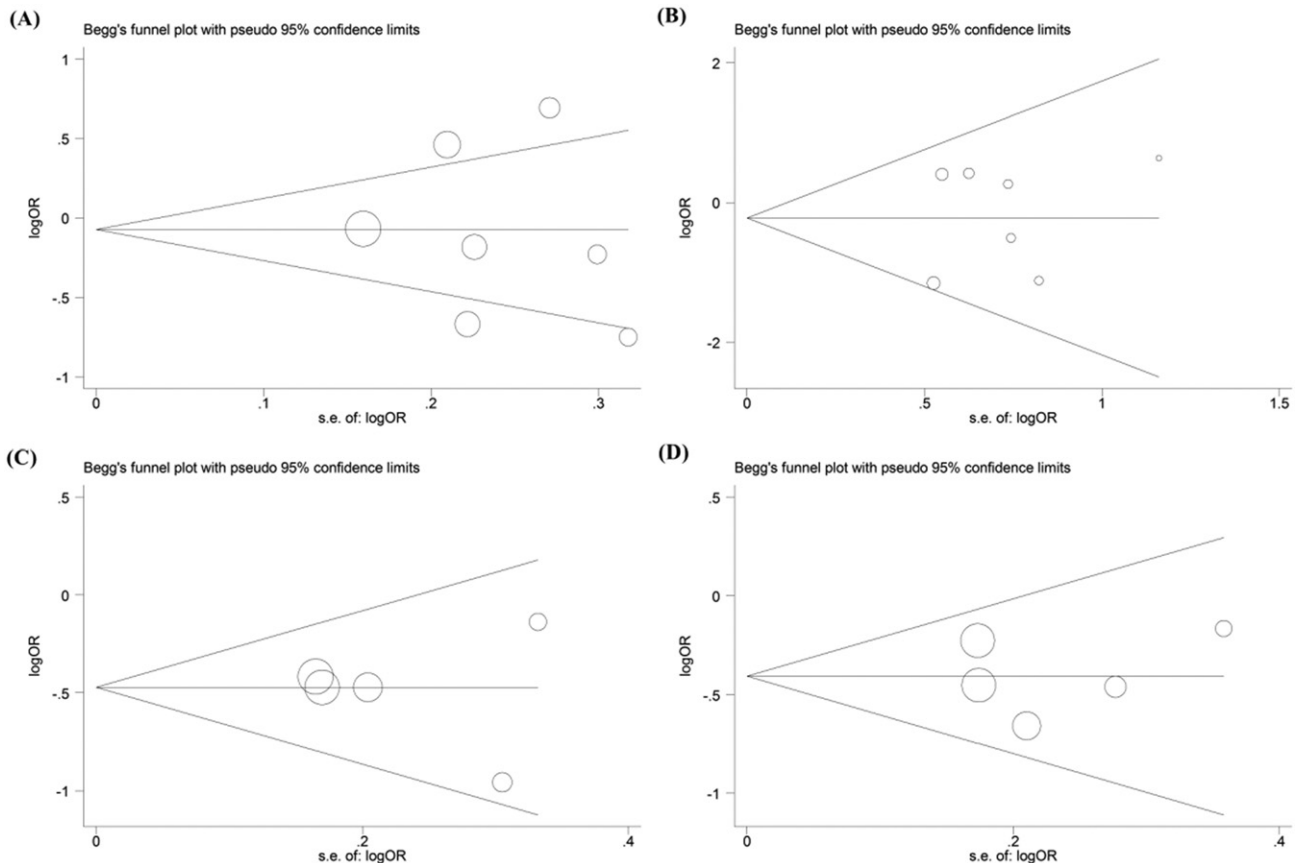
asymmetry on the natural logarithm scale of the OR. Significance sets at  $P_E < 0.05$ .

In our results, all funnel plots showed no obvious asymmetry from visual inspection (Fig. 4). Egger's regression test also confirmed that no publication bias existed.  $P_E$  values for 137G/C polymorphism were 0.831, 0.873, 0.635, 0.760 and 0.707, respectively.  $P_E$  values for 607C/A polymorphism were 0.989, 0.887, 0.742, 0.756 and 0.918, respectively.

**Table 2**  
The main results of the meta-analysis.

Study group	Sample size (case/control)	Allelic model (d allele vs. D allele)		Additive model (dd vs. DD)		Additive model (Dd vs. DD)		Dominant model (dd + Dd vs. DD)		Recessive model (dd vs. Dd + DD)	
		OR [95%CI]	p	OR [95%CI]	p	OR [95%CI]	p	OR [95%CI]	p	OR [95%CI]	p
<i>Overall analysis</i>											
137G/C	1566/1372	0.91[0.66.1.25]	0.55	0.79[0.50.1.27]	0.33	0.92[0.64.1.32]	0.64	0.91[0.63.1.31]	0.61	0.78[0.49.1.24]	0.29
607C/A	1391/1194	0.73[0.65.0.81]	<0.01	0.51[0.40.0.64]	<0.01	0.68[0.56.0.82]	<0.01	0.62[0.52.0.74]	<0.01	0.67[0.55.0.80]	<0.01
<i>Subgroup analysis based on types of ischemic stroke</i>											
137G/C (LAA)	267/602	0.55[0.40.0.77]	0.01	0.20[0.05.0.85]	0.03	0.56[0.28.1.11]	0.10	0.53[0.30.0.94]	0.03	0.22[0.05.0.94]	0.04
137G/C (SVD)	295/602	0.79[0.60.1.05]	0.10	0.39[0.13.1.15]	0.09	0.84[0.50.1.42]	0.52	0.79[0.48.1.29]	0.35	0.40[0.14.1.18]	0.10
137G/C (CE)	73/602	1.17[0.55.2.49]	0.68	0.69[0.13.3.70]	0.66	1.47[0.56.3.81]	0.43	1.34[0.52.3.45]	0.54	0.58[0.11.3.11]	0.53
607C/A (LAA)	183/384	0.52[0.40.0.67]	<0.01	0.27[0.16.0.47]	<0.01	0.41[0.27.0.61]	<0.01	0.37[0.25.0.54]	<0.01	0.50[0.31.0.80]	<0.01
607C/A (SVD)	189/384	0.87[0.68.1.11]	0.26	0.20[0.12.0.32]	<0.01	1.41[0.89.2.20]	0.15	1.18[0.76.1.83]	0.47	0.54[0.34.0.86]	<0.01
607C/A (CE)	51/384	1.32[0.87.2.01]	0.19	0.73[0.37.1.44]	0.36	0.86[0.39.1.90]	0.71	1.10[0.53.2.28]	0.81	1.78[0.97.3.29]	0.06
<i>Subgroup analysis based on age of subjects</i>											
137G/C (~65)	160/154	1.15[0.73.1.79]	0.55	0.34[0.04.3.34]	0.36	1.34[0.81.2.22]	0.26	1.26[0.77.2.08]	0.36	0.32[0.03.3.08]	0.32
137G/C (65~)	263/230	0.71[0.50.1.02]	0.06	0.30[0.09.0.95]	0.04	0.86[0.56.1.31]	0.49	0.77[0.51.1.15]	0.20	0.31[0.10.0.98]	0.05
607C/A (~65)	319/317	0.57[0.45.0.71]	<0.01	0.36[0.22.0.58]	<0.01	0.50[0.35.0.72]	<0.01	0.46[0.33.0.64]	<0.01	0.49[0.32.0.77]	<0.01
607C/A (65~)	227/201	0.89[0.68.1.18]	0.42	0.77[0.38.1.56]	0.47	0.93[0.48.1.81]	0.83	0.87[0.46.1.67]	0.68	0.82[0.54.1.24]	0.35
<i>Subgroup analysis based on gender of subjects</i>											
137G/C (Male)	256/231	0.76[0.53.1.09]	0.14	0.10[0.01.0.77]	0.03	0.98[0.64.1.49]	0.92	0.85[0.57.1.28]	0.44	0.10[0.01.0.77]	0.03
137G/C (Female)	167/153	1.02[0.66.1.56]	0.94	0.75[0.20.2.85]	0.67	1.11[0.67.1.84]	0.69	1.07[0.65.1.74]	0.80	0.73[0.19.2.76]	0.64
607C/A (Male)	526/464	0.65[0.55.0.78]	<0.01	0.39[0.27.0.58]	<0.01	0.75[0.56.1.01]	0.05	0.63[0.48.0.84]	<0.01	0.46[0.33.0.64]	<0.01
607C/A (Female)	116/131	0.97[0.67.1.40]	0.87	0.72[0.33.1.59]	0.42	0.52[0.24.1.15]	0.10	0.61[0.29.1.28]	0.19	1.19[0.72.1.98]	0.50
<i>Sensitivity analysis</i>											
137G/C (High quality)	1413/1258	0.82[0.61.1.11]	0.20	0.78[0.40.1.13]	0.13	0.82[0.58.1.16]	0.26	0.81[0.57.1.14]	0.23	0.69[0.41.1.15]	0.15
607C/A (HWE $p_H > 0.05$ )	1198/1074	0.74[0.66.0.83]	<0.01	0.53[0.42.0.68]	<0.01	0.72[0.58.0.88]	<0.01	0.66[0.54.0.79]	<0.01	0.67[0.55.0.82]	<0.01

d was for the mutant allele and D was for the common allele; OR, odds ratio; CI, confidence intervals; p, a p-value of the test; LAA, large artery atherosclerosis; SVD, small vessel disease; CE cardioembolism; High quality, NOS (Newcastle Ottawa Scale) score > 7; HWE, Hardy-Weinberg equilibrium.



**Fig. 4.** Begg's funnel plots for IL-18 promoter 137G/C polymorphism and risk of ischemic stroke in the genetic models of CC + GC vs. GG (A) and CC vs. GC + GG (B), and 607C/A polymorphism in the genetic models of AA + CA vs. CC (C) and AA vs. CA + CC (D).

#### 4. Discussion

IS is one of the leading causes of morbidity and mortality worldwide. The etiology of IS is complex and poorly defined. Accumulating evidence suggests that inflammatory cytokines exert important role in the pathogenesis of IS (Fann et al., 2013). Reportedly, IL-18 is a pleiotropic pro-inflammatory cytokine regulating both innate and acquired immunity, and inflammatory processes (Okamura et al., 1998). Gene polymorphisms of some inflammation-related genes including IL-6 rs1800796, transforming growth factor-beta1 (TGF- $\beta$ 1) T869C polymorphism and tumor necrosis factor beta (TNF- $\beta$ ) rs909253 have been reported to be related to risk of IS (Peng et al., 2013; Wang et al., 2014; de Sousa Parreira et al., 2015). Some single-center studies have reported that the two polymorphisms of the IL-18 gene promoter, i.e., 137G/C and 607C/A, were associated with IS risk, although the results were inconsistent (Chen et al., 2010; Zhang et al., 2010; Li et al., 2011; Wang and Cheng, 2011; Ren et al., 2012; Lu et al., 2013; Wang et al., 2013; Wei et al., 2013). Overall, the present meta-analysis provided systematic analysis on the relationship between IL-18 gene promoter polymorphisms (137G/C and 607C/A) and risk of IS in the Chinese population.

Our data showed that IL-18 gene promoter 607A polymorphism, but not 137C polymorphism, was a protective factor for IS. In the pooled results, individuals carrying C-allele (607C/A) had approximately 1.37-fold higher risk for developing IS than those with A-allele. In addition, the IS risk of AA and CA carriers was significantly lower compared with CC carriers (OR = 0.51 and 0.68, respectively). Subgroup analyses were conducted based on the subtypes of ischemic stroke (LAA, SVD and CE), age of subjects (younger and older than age 65) or sex of subjects (male and female). The protective association of 607A polymorphism was stronger in the subgroups of male subjects and subjects younger than age 65, and weaker in females and individuals older than age 65. Also, the protective association of 607A polymorphism was stronger in subjects with LAA than in subjects with SVD. Because LAA is caused by atherosclerotic plaques, and the effects of IL-18 on arteriosclerotic plaque stability is convincingly established (Mallat et al., 2001a; de Nooijer et al., 2004; Bouki et al., 2012), the IL-18 607A allele is an important factor that determines the risk of IS in LAA individuals. In contrast, SVD is not manifested by atherosclerotic plaques and IL-18 polymorphisms play minimal, if any, risk factor in SVD individuals. Moreover, in subgroup of subjects with SVD, the 607AA homozygote, but not CA heterozygote, could contribute to reduced risk of IS. This probably suggests that the development of IS derived from the large arteries differs from that in the small vessels. Men comprise the major component of alcohol abusers, smokers, and people with unhealthy lifestyle; therefore, men may be at greater risk of IS than women, and may be influenced by IL-18 polymorphism more profoundly. One previous study reported that genetic susceptibility to atherosclerosis decreases with age (Ilveskoski et al., 1999). A plausible explanation is that accumulation of environmental risk factors and phenocopies in elders dilute the effect of gene polymorphism in the pathogenic cascade. These results can explain why the protective effects of 607 A polymorphism are manifested better in the younger than in the older age subgroup. For 137G/C polymorphism, C-allele and CC genotype were associated with lower risk of LAA. However, only CC homozygote played a potential protective effect on IS in men and in subjects older than 65. The interpretation of our results is also affected by heterogeneity. In CE subtypes, we established no significant differences between these the 607C/A and 137G/C promoter polymorphisms and risk of IS. This could be due to the small sample size we had available for meta-analysis, or a more complex effects of IL-18 polymorphism on CE subtype individuals.

Furthermore, the corresponding pooled ORs were not substantively altered after restricting the included studies in agreement with HWE or with NOS score > 7, indicating that our results were robust and reliable.

In the present study, regarding 137G/C polymorphism, heterogeneity existed both in the overall comparisons and subgroup analyses. Subgroup analyses and sensitivity analyses also helped clarify the source of the heterogeneity. Regrettably, we failed to confirm the sources of heterogeneity. Some factors such as sampling error, diversity in design or the interaction with other risk factors (genetic or environmental) may be the cause to this heterogeneity.

IL-18 is expressed in human atherosclerotic plaques and contributes to plaque destabilization through increasing the expression of cytokines and cellular adhesion molecules such as IL-1 $\beta$ , IL-8 and vascular cell adhesion molecule-1 (Mallat et al., 2001a). In apoE deficient mice, exogenous IL-18 administration promotes atherosclerotic plaque progression and destabilization through enhancing the inflammatory process (Whitman, 2002; Bhat et al., 2015). Murine IL-18 binding protein (IL-18BP), the endogenous inhibitor of IL-18, induces a stable plaque phenotype by reducing macrophage infiltration, T lymphocyte count, cell death, and total lipid content, and increasing smooth muscle cell accumulation and cellular collagen content (Mallat et al., 2001b). In addition, IL-18 deficient apoE knockout mice showed compatible with a more stable atherosclerotic lesion phenotype compared to control apoE knockout mice (Elhage, 2003).

Atherosclerosis is a chronic, low grade inflammatory process, which may cause an increased risk of stroke. IL-18-induced inflammatory responses play an important role during stroke. Thus, within the first 24 h of onset, IS patients have higher serum concentrations of IL-18 than healthy controls (Zaremba and Losy, 2003). Also, higher IL-18 serum levels correlate with more severe neurological stroke and functional disability (Piazza et al., 2010; Ormstad et al., 2011). In a prospective cohort study from Taiwan, the group of patients with plasma IL-18 levels higher than 780 pg/mL showed significantly higher incidence of 90-day recurrent stroke and 90-day accumulative death (Yuen et al., 2007). A delayed increase of IL-18 mRNA was observed 48 h post rat cerebral ischemia, which reached its peak between 7 and 14 days (Jander et al., 2002). These and other similar studies suggest that circulating IL-18 levels may be an indicator of stroke outcome. However, previous studies have failed establishing reliable correlation between the IL-18 levels and the severity of IS in humans and mice, thus leaving the issue unsettled at the present stage (Wheeler et al., 2003; Stott et al., 2009; Jefferis et al., 2013).

Some limitations should be addressed in this meta-analysis as well. Firstly, heterogeneity may affect the interpretation of our results. Of all reviewed studies, only three (Zhang et al., 2010; Wang and Cheng, 2011; Lu et al., 2013) performed the subgroup analyses based on the subtypes of IS, age or sex of subjects. Due to the small number of reports and subgroups (e.g., sex, age or other relevant factors such as smoking and drinking), the accuracy the meta-analysis data is inherently poor. In addition, some inevitable bias existed in our results due to the limitations of inclusion criteria, although Begg's funnel plots and Egger's regression test showed no publication bias. Furthermore, power calculations for the given sample size of 137G/C polymorphism demonstrated that the meta-analysis was underpowered. Therefore, the data of our study should be interpreted with caution, especially involving 137G/C polymorphism.

In conclusion, this is the first meta-analysis investigating the association of IL-18 gene promoter polymorphisms and IS risk. Our data suggest that IL-18 gene promoter 607C/A polymorphism is a protective factor for IS in the Chinese population, while 137G/C polymorphism has weaker or no protective properties. Still, a larger number of studies with large scale and sufficient original information are required to further confirm our findings.

#### Conflict of interest

The authors have declared that no competing interests exist.

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Checklist S1 PRISMA 2009 Checklist.

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