Association between Lymphotoxin Alpha (-252G/A and -804C/A) Gene Polymorphisms and Risk of Ischemic Stroke: A Meta-Analysis

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Abstract

Purpose: Lymphotoxin-Alpha (LTA) is a mediator of inflammation which may be associated with the risk of ischemic stroke (IS). Polymorphisms (-252A/G and -804C/A) in the LTA gene have been found to be associated with IS with contradictory results. The present meta-analysis aimed to provide a comprehensive account of the association of (-252A/G and -804C/A) gene polymorphisms of LTA gene with susceptibility to IS.

Methods: A literature search for eligible candidate gene studies published before April 20, 2015 was conducted in the PubMed, EMBASE, Trip database and Google Scholar. The following combinations of main keywords were used: ('Lymphotoxin-alpha' or 'LTA' or 'tumour necrosis factor beta' or 'TNF-beta') and ('Ischemic stroke or 'cerebral infarction' or 'IS') and ('genetic polymorphism' or 'single nucleotide polymorphisms' or 'SNP'). Fixed or random effects models were used to estimate the strength of association through Odds ratios (ORs) and 95% confidence interval (CI).

Results: Four case-control studies for LTA -252A/G gene polymorphism showed no significant association under; dominant (OR, 0.9; 95% CI; 0.8 to 1.0, P value 0.34), recessive (OR, 1.1; 95% CI; 0.9 to 1.3; P value 0.21) models, indicating that GG and AG genotypes may not possibly confer an increased susceptibility to IS as compared to AA genotype. For LTA -804C/A gene polymorphism, three case-control studies also showed no significant association under; dominant (OR, 0.5; 95% CI; 0.1 to 2.3; P value 0.44), recessive (OR, 0.8; 95% CI; 0.38 to 2.07, P value 0.79) models with IS risk.

Conclusion: Based on ethnicity stratification, our meta-analysis suggests that LTA -252A/G gene polymorphism is found to be significantly associated with the risk of IS in Caucasian population, but not in Asian population. However, LTA -804C/A gene polymorphism is not found to be associated with the susceptibility of IS in both Asian as well as in Caucasian population. Further well designed large sample size prospective studies are needed to confirm these findings.

Key Words: Lymphotoxin-alpha, Cytokine, Tumour Necrosis factor-beta, Cerebral infarction, ischemic stroke, Meta-analysis.

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INTRODUCTION

Ischemic Stroke (IS) is a complex multifactorial disease caused by the combination of vascular risk factors, environmental and genetic factors[1]. Inflammation and genetics are both prominent mechanisms in the pathogenesis of IS[2]. A number of cytokine genes have been consistently reported to be associated with the risk of IS. A growing body of evidence suggests that Lymphotoxin-α (LTA) is the predominant member of the tumor necrosis factor (TNF) ligand family, which elicits pro-atherogenic responses and plays an important role in the pathogenesis of IS. Lymphotoxin alpha (LTA) is one of the proinflammatory cytokines which is produced mainly by lymphocytes in response to tissue injury. The Human LTA gene is located on short arm of chromosome 6 (6p21) and is found to be expressed in atherosclerotic plaques[3,4]. LTA can induce adhesion molecules and cytokines from vascular endothelial, vascular smooth muscle cells and certain types of leukocytes; these substances are expressed in atherosclerotic plaques, and their levels in circulation are elevated in atherosclerosis and cerebrovascular diseases, including stroke[5-7].

Studies have confirmed that polymorphism at 252 positions (A/G) in intron 1 increases the transcriptional activities of LTA gene and functional polymorphism at 804 (C/A) position in exon 3 of LTA gene leads to a change in the amino acid threonine (T) to asparagine (N) at codon 26 which promotes post-ischemic inflammation[4,8-11]. The presence of these SNPs therefore could be postulated as ultimately promoting atherogenetic processes in general. Limited number of studies have been performed to investigate the association of rs909253 (-252A/G) or rs1041981 (-804C/A) with the risk of IS with conflicting results which may be due to small sample size and differences in the study populations[8,12-16]. Meta-analysis is a useful approach for investigating associations between genetic factors and disease, because a quantitative approach is used to combine the results from different studies on the same topic, thereby providing more reliable conclusions. Therefore, we performed a meta-analysis to investigate a comprehensive account of the association of LTA gene polymorphisms (-252A/G and -804C/A) with the susceptibility to IS.

MATERIALS AND METHODS

Identification of Relevant Studies

A literature search for genetic polymorphism studies that investigated the association between the LTA gene polymorphisms and susceptibility to IS published before April 20, 2015 was conducted in the following electronic databases: PubMed, EMBASE Google Scholar and Trip database. The following combinations of main keywords were used: ('Lymphotoxin-alpha' or 'LTA' or 'tumour necrosis factor beta' or 'TNF-beta') and ('Ischemic stroke or 'cerebral infarction' or 'IS') and ('genetic polymorphism' or 'single nucleotide polymorphisms' or 'SNP'). The search was done in any language, but only included those studies that were conducted on human subjects. All references in eligible articles were extensively reviewed to identify additional published articles.

INCLUSION AND EXCLUSION CRITERIA

To be included in the analysis, eligible studies have to meet the following criteria: (1) case-control studies on the association between LTA gene polymorphism and IS; (2) all patients in the candidate studies meet the diagnostic criteria for IS; (3) studies with sufficient available data to calculate ORs with corresponding 95%CIs. The major reasons for excluding studies were: (1) not a case-control study; (2) duplicate publications with overlapping subjects from the same study; and (3) no available data reported. This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guideline[17]. No author was contacted regarding the missing information that was required for the meta-analysis to avoid the risk of retrieval bias.

DATA EXTRACTION

According to the PRISMA guideline, two investigators PK and SM independently checked each full-text report for eligibility and extracted the following data from eligible studies: surname of first author, year of publication, country of origin, ethnicity, definition and number of case and control, age, sex ratio, genotyping method, genotype

frequency, etc. Disagreements were solved by discussion between all authors until consensus was reached.

QUALITY ASSESSMENT

We also evaluated the methodological quality of each study which is included in our analysis using a quality assessment scale[18] developed for genetic association studies which was modified by us to increase the relevance of our study. This scale took into account both traditional epidemiological considerations and genetic issues. The scores ranged from 0 (worst) to 16 (best). Details of the scale are presented in Table-1. Two authors independently assessed the quality of included studies. Discrepancies over quality scores were resolved by discussion with all authors and subsequent consensus was reached.

Table 1. Scale for quality assessment

Criteria	Score
Representativeness of cases	
Selected from any population disease registry or multiple center sites	2
Selected from any cardiology/neurology	1
Not described	0
Source of controls	
Population or neighbour based	3
Hospital based	2
Healthy volunteers with total description	1
Healthy volunteers without total description	0.5
Not described	0
Matching of Controls	
Age & Sex match	2
Smoking, hypertensive, Diabetics	1
Not matched	0
Ascertainment of IS	
Adequate confirmation	2
Diagnosis of IS by patient medical record	1
Not described	0
Ascertainment of Controls	
Stroke frees status by using appropriate QVSS or CT/MRI	1
Not described	0
Genotyping	
Genotyping done under blinded conditions	1
Unblinded or not mentioned	0
Genotyping Method	
DNA sequencing/Multiplex PCR	2
PCR-RFLP	1
Others	0
HWE	
Allelic frequency in accordance HWE	2
Not HWE but followed statistics to adjust confounding	1
Not Checked	0
Association assessment	
Appropriate statistics and examining confounders and effect modifiers	1
Inappropriate statistics used Inappropriate statistics used	0
Total Score	16

STATISTICAL ANALYSIS

Hardy Weinberg Equilibrium (HWE) in the controls was tested by comparing the expected and observed genotype frequencies using chi-square test. The association between the LTA genetic polymorphisms and susceptibility to IS was assessed by the pooled odds ratios (ORs) with their corresponding 95% confidence intervals (95% CIs) under two genetic models, including dominant and recessive model. Heterogeneity was assessed by using Cochran's Q statistic and I2 metric[19]. In our study, the I2 values exceeding 50% and heterogeneity at the 10% level of significance were considered as an indicator of significant heterogeneity. Fixed effects model was used to estimate the OR and 95% CI if heterogeneity was <50% otherwise random effects model was used. The software

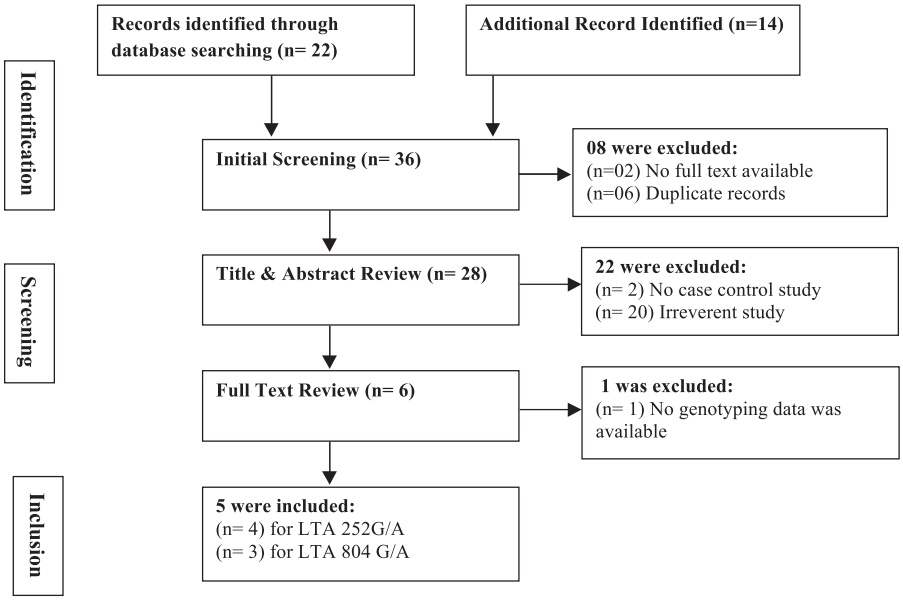


Figure 1: Flow diagram of the selection of studies and specific reasons for exclusion from the present meta-analysis.

Table 2. Characteristic of studies included in the meta-analysis of the association of LTA gene polymorphism with the risk of ischemic stroke

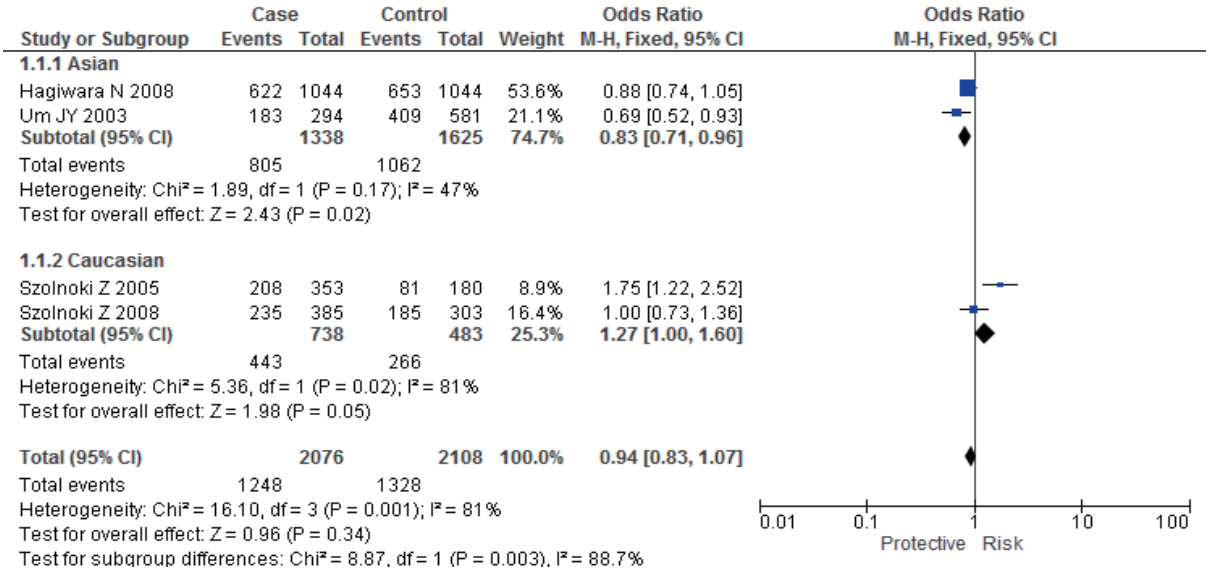
S.No	Year	Author	Origin	Ethnicity	Cases/ controls	HWE	Matching criteria	Genotyping Method	Variants	M/F	Age [Mean ± S.D]	Quality Score	Source of control
1	2003	Um *et al.*[13]	Korea	Asian	294/ 581	No	Age + Sex	PCR-RFLP	-252A/G	209/136	61.0 ± 14.5	9	HB
										78/83	65.4±9.5		
2	2005	Szolnoki *et al.*[11]	Hungary	Caucasian	353/ 180	No	Sex	PCR-RFLP	-252A/G -804C/A	190/163	63.5 ± 12.1	9	HB
										98/82	55.6 ±12.3		
3	2008	Hagiwara *et al.*[14]	Japan	Asian	1044/ 1044	Yes	Age + Sex	RT-PCR	-252A/G -804C/A	656/388	69.9 ± 9.8	11	PB
										656/388	69.8 ± 9.9		
4	2009	Szolnoki *et al.*[16]	Hungary	Caucasian	385/ 303	Yes	NA	PCR-RFLP	-252A/G	222/163	67.4±13.65	9	HB
										201/102	57.4±14.3		
5	2009	Freilinger *et al.*[15]	Germany	Caucasian	601/ 736	Yes	Age + Sex	MALDI-TOF	-804C/A	377/-	64/-	10	HB
										447/-	62/-		

used for carrying out the meta-analysis was Review Manager (version 5.3; Cochrane Collaboration, Syracuse, NY, USA).

RESULTS

A total of 36 research articles were identified using the pre-specified search strategy. Figure-1 presents a flow diagram of retrieved and excluded studies with their reasons for exclusion. In accordance with the inclusion criteria, five case-control studies were included in our meta-analysis. Of the included studies, four studies involving 2076 cases and 2108 controls examined association of LTA -252A/G and three studies involving 1998 cases and 1960 controls examined association of -804C/A with the risk of IS. Studies were conducted in two major ethnic populations, with two on Asians and three on Caucasians. The publication years of included

A. Dominant Model LTA -252A/G



B. Recessive Model LTA -252A/G

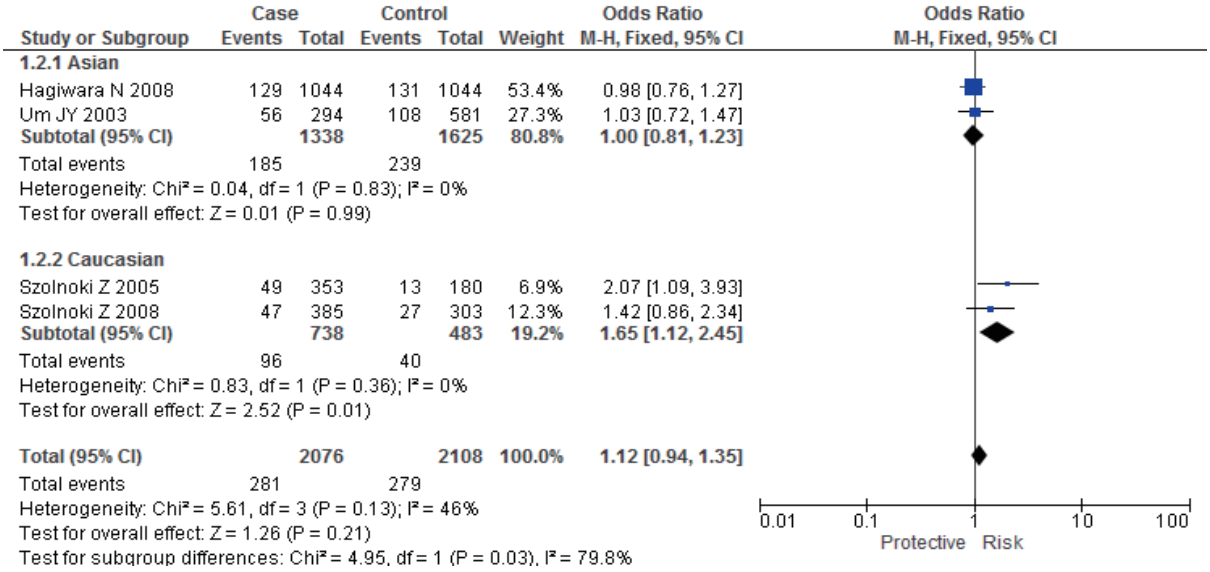
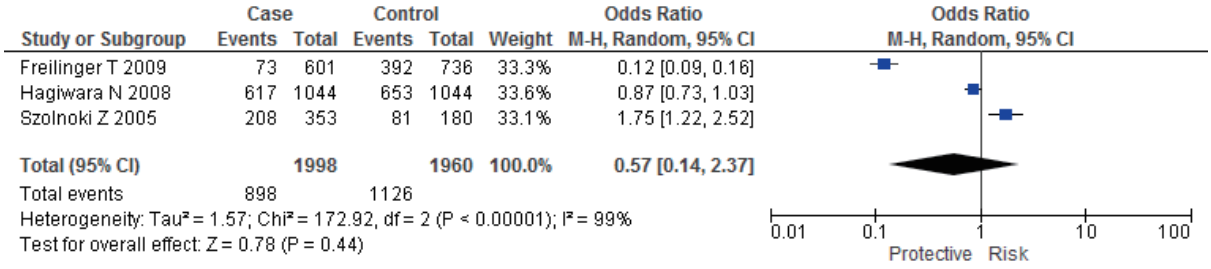


Figure 2: B. Recessive Model LTA -252A/G

A. Dominant Model -804C/A



B. Recessive Model LTA -804C/A

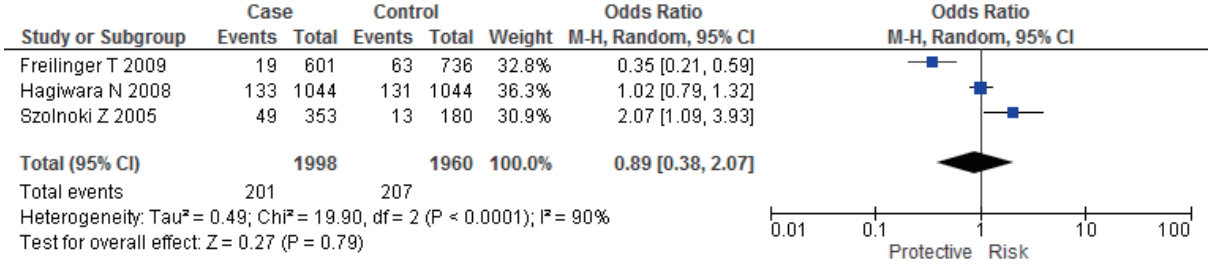


Figure 3: Forest plots of ORs for the association between the LTA -804C/A polymorphism and susceptibility to Ischemic stroke (A) Dominant model [AA vs. CC+CA] (B) Recessive Model [CA+AA vs. CC]

studies ranged from 2003 to 2009. Three studies in this meta-analysis had controls in accordance to HWE. The quality scores of all included studies were moderately high. The characteristics and methodological quality of all the included studies are summarized in Table-2.

Association between -252A/G polymorphism of LTA gene and IS

LTA -252A/G gene polymorphism was assessed in 4 case-control studies with a total of 2076 IS cases and 2108 controls. No significant association was found under all models; dominant (GG vs. AA+AG: OR, 0.9; 95% CI; 0.8 to 1.0; P value 0.34), recessive (AG + GG vs. AA: OR, 1.1; 95% CI; 0.9 to 1.3; P value 0.21) indicating that GG and AG genotypes may not possibly confer an increased susceptibility to IS compared to AA genotype [Figure-2]. A significant heterogeneity was observed under dominant model (x^2= 16.10; p_{Het}= 0.001). No significant heterogeneity was observed under recessive model (x^2= 5.61; p_{Het}= 0.13).

After stratified analysis based on ethnicity, 2 studies involving 738 IS cases and 483 controls showed significant association with IS risk under dominant (GG vs. AA+AG: OR,1.2; 95% CI; 1.0 to 1.6; P value 0.05], recessive (AG + GG vs. AA: OR, 1.6; 95% CI; 1.1 to 2.4; P value 0.01) in Caucasian population. However, 2 studies involving 1338 IS cases and 1625 controls showed protective mode of association under dominant (GG vs. AA+AG: OR, 0.8; 95% CI; 0.7 to 0.9; P value 0.02) and no significant association with IS risk was observed under recessive model (AG + GG vs. AA: OR, 1.0; 95% CI; 0.8 to 1.2; P value 0.99) in Asian population.

Association between -804C/A polymorphism of LTA gene and IS

Three case-control studies had investigated the relationship between -804C/A and susceptibility to IS with a total of 1998 IS cases and 1960 control subjects. For LTA -804C/A, no significant association was observed under all models; dominant (AA vs. CC+CA: OR, 0.5; 95% CI; 0.1 to 2.3; P value 0.44), recessive (CA+AA vs. CC: OR, 0.8; 95% CI; 0.3 to 2.0; P value 0.79) models with IS risk (Figure-3).

DISCUSSION

A number of meta-analyses have been done to investigate the relationship between the LTA gene and various diseases, including gastric cancer[20], breast cancer[21] and Myocardial infarction[22]. To the best of our knowledge, our study is the first meta-analysis to describe genetic polymorphisms in LTA gene with the susceptibility to IS. This meta-analysis provides a comprehensive summary of the currently available evidence on the associations between the LTA -252A/G and -804C/A polymorphisms and susceptibility to IS. Our meta-analysis of five studies suggests that -252A/G LTA gene polymorphism is significantly associated with risk of IS in Caucasian population. However, LTA -804C/A gene polymorphism is not found to be associated with the risk of IS.

The limitations of our meta-analysis includes: (1) less number of studies; (2) summarizes the findings of only two SNPs of a single gene; (3) inadequate power to draw solid conclusion; and (4) heterogeneity was a major concern when interpreting the results of meta-analysis. We observed more heterogeneity in our findings as individuals of different ethnicities may have diverse genetic back grounds and environmental factors, and as a result the same polymorphism may play different roles in different populations. In our meta-analysis, studies were conducted in two major ethnic populations, with two in Asian and three in Caucasian. Three studies in this meta-analysis had controls in accordance to HWE. The source of control in four studies was Hospital based and in one study was Population based. In addition, the source of control was another factor that contributed to heterogeneity. The genotype distribution in population-based controls may be similar to normal and thus population-based controls could be more reliable than hospital based controls. However, both population-based and functional studies are required to clarify the susceptibility of LTA gene polymorphisms to ischemic stroke.

CONCLUSION

Our meta-analysis suggests that LTA -252A/G gene polymorphism is found to be significantly associated with the risk of IS in Caucasian population, but not in Asian population. However, LTA -804C/A gene polymorphism is found not to be significantly associated with the susceptibility of IS in both Asian as well as Caucasian population. Further well designed large sample size studies with population based controls are needed to confirm these findings.

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