

C-reactive Protein –717A>G and –286C>T>A Gene Polymorphism and Ischemic Stroke

Yan Liu¹, Pei-Liang Geng², Fu-Qin Yan³, Tong Chen¹, Wei Wang¹, Xu-Dong Tang⁴, Jing-Chen Zheng⁵, Wei-Ping Wu¹, Zhen-Fu Wang¹

¹Department of Nanlou Neurology, The General Hospital of PLA, Beijing 100853, China

²Department of Cancer Center, The General Hospital of PLA, Beijing 100853, China

³Department of Pharmacy, The General Hospital of Chinese People's Armed Police Forces, Beijing 100039, China

⁴Department of Gastroenterology, Xiyuan Hospital, Beijing 100091, China

⁵Department of Institute of Disaster Relief Medical Science, The General Hospital of Chinese People's Armed Police Forces, Beijing 100039, China

Abstract

Background: Inflammation plays a pivotal role in the formation and progression of ischemic stroke. Recently, more and more epidemiological studies have focused on the association between C-reactive protein (CRP) –717A > G and –286C > T > A genetic polymorphisms and ischemic stroke. However, the findings of these researches are not conclusive.

Methods: We performed a meta-analysis to determine whether these two polymorphisms are associated with the risk of ischemic stroke. Eligible studies were identified from the database of PubMed, Medline, Embase, Web of Science, CNKI, Weipu, and Wanfang. We calculated odds ratios (ORs) with 95% confidence intervals (CIs) to assess the strength of the association.

Results: Four articles were included in our study, including 1926 cases and 2678 controls for –717A > G polymorphism, 652 cases and 1103 controls for –286C > T > A polymorphism. The results of meta-analysis showed that single nucleotide polymorphism (SNP) –717A > G was not significantly associated with the risk of ischemic stroke (GG vs. AA, OR = 1.12, 95% CI = 0.83–1.50, P = 0.207; GG + GA vs. AA, OR = 1.04, 95% CI = 0.93–1.17, P = 0.533; GG vs. GA + AA, OR = 1.10, 95% CI = 0.82–1.47, P = 0.220). Meta-analysis of SNP –286C > T > A also demonstrated no statistical evidence of a significant association with the risk of ischemic stroke (AA vs. CC, OR = 0.86, 95% CI = 0.59–1.25, P = 0.348; AA vs. CC, OR = 0.92, 95% CI = 0.80–1.06, P = 0.609; AA vs. CC, OR = 0.89, 95% CI = 0.62–1.30, P = 0.374).

Conclusions: This meta-analysis demonstrated little evidence to support a role of CRP gene –717A > G, –286C > T > A polymorphisms in ischemic stroke predisposition. However, to draw comprehensive and more reliable conclusions, further larger studies are needed to validate the association between CRP gene polymorphisms and ischemic stroke in various ethnic groups.

Key words: C-reactive Protein; Polymorphism; Ischemic Stroke; Meta-analysis

INTRODUCTION

Ischemic stroke constitutes approximately 85% of all strokes and has caused a large number of deaths globally.^[1,2] Much effort has been taken over the past decade to gain new insights into the pathogenesis of ischemic stroke, with a large number of genetic markers discovered via family and twins studies.^[3,4] Since then many epidemiologic and molecular studies have emerged to test the hypothesis that genetic variations may be associated with a predisposition to this heterogeneous disease.

Inflammation plays a pivotal role in the formation and progression of ischemic stroke, which represents an

important causative factor for ischemic stroke.^[5,6] C-reactive protein (CRP, gene ID: 1401) belongs to the pentraxin protein family and functions through Fc-gamma receptors to defend against inflammatory responses and autoimmune diseases.^[7] There is mounting evidence of serum CRP level as a predictor of ischemic stroke.^[8–10] The human CRP gene with a spanning size of 2880 bps is mapped at chromosome 1 at q21–q23. The magnitude of CRP expression is genetically determined, and almost half of the alternation depends on host factors.^[11,12] As CRP gene variations involves in the regulation of the protein level,^[13] investigating the association of the single nucleotide polymorphisms (SNPs) and ischemic stroke susceptibility may have diagnostic and prognostic implications.

The promoter SNPs in CRP gene, CRP –717A > G and –286C > T > A gene polymorphisms, were proposed as possible biomarkers and suggested to be used to

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Address for correspondence: Dr. Zhen-Fu Wang,

Department of Nanlou Neurology, The General Hospital of PLA,
Beijing 100853, China

E-Mail: wangzf301@126.com

predict ischemic stroke in some researches.^[14,15] However other studies showed that the development of ischemic stroke should not attribute to CRP SNPs.^[16-18] In view of the inconsistent reports, we undertook a meta-analysis to determine whether the risk of ischemic stroke is associated with CRP -717A > G and -286C > T > A gene polymorphisms.

METHODS

Literature retrieval strategy

To identify eligible studies on the association between CRP -717A > G and -286C > T > A genetic polymorphisms and ischemic stroke, we performed a literature search of published studies in PubMed, Medline, Embase, Web of Science, CNKI database, Weipu database, and Wanfang database from 1980 to April 2014. The keywords we used are as follows: CRP or C-reactive protein, polymorphism or polymorphisms or genetic variants, as well as ischemic stroke. Besides, we inspected references of all identified studies for further relevant studies.

Selection criteria

Studies were included in the meta-analysis if they met all of the following criteria: (1) Used a case-control design or cohort design; (2) investigated the association between CRP -717A > G and -286C > T > A gene polymorphisms and ischemic stroke risk; (3) contained sufficient data which could help to calculate an odds ratio and 95% confidence intervals (*OR* and 95% *CI*s); (4) genotype distribution in controls was in Hardy-Weinberg equilibrium (HWE).

Data extraction

Two authors independently extracted data from the included studies onto the standard, data-collection forms. To facilitate a general understanding of the eligible studies, in addition to allele and genotype data, two investigators also extracted the first author's surname, publication year, subjects' ethnicity, matching, control sources, DNA source, number of cases and controls, *P* value for HWE wherever possible, and measurement methods in duplicate. If there were disagreements on conflicting data, discussion among all investigators would be held to solve them.

Statistical analysis

Based on the genotype data in controls, we tested HWE for all studies using the goodness-of-fit Chi-square test. The association between CRP SNPs and ischemic stroke risk was evaluated using crude *OR* with 95% *CI*. Summary *OR*s were calculated assuming homozygote, dominant and recessive contrast models for both SNPs.

Inconsistency across studies (heterogeneity) was determined with the Chi-square-based *Q*-test and the *I*² metric.^[19] If *P* values above 0.10 or *I*² more than 50%, which means significant heterogeneity, we appropriately selected the random-effects model (Mantel and Haenszel method) to calculate the effects size; otherwise, the fixed-effects model (DerSimonian and Laird method) was performed.^[20,21]

Sensitivity analysis, Egger's test and Begg's funnel plot were performed respectively to examine the stability and reliability of the combined effects in this meta-analysis.^[22-24] All statistical data were analyzed with STATA software (version 12.0, STATA Corp., College Station, TX, USA). All two-tailed *P* values were considered as significant at 0.10 unless specially stated.

RESULTS

Literature retrieval

A total of 461 records matching the search terms were obtained from the PubMed, Medline, Embase, Web of Science, CNKI database, Weipu database, and Wanfang database. Through carefully reading the titles and abstracts, four potential articles were identified.^[14-17] According to the above-mentioned criteria, we excluded one publication, which was not designed as case-control or cohort.^[14] Another study was identified through manual search.^[18] Finally, four articles met the inclusion criteria and were included in the meta-analysis.

Characteristics of the studies

For the -717A > G polymorphism, 1926 ischemia stroke cases and 2678 controls from four articles were identified to estimate the relationship between the polymorphism and ischemia stroke risk.^[15-18] For the -286C > T > A polymorphism, 652 ischemia stroke cases and 1103 controls from three articles were analyzed to assess the association between the polymorphism and ischemia stroke risk.^[15,16,18] For the studies finally considered in this meta-analysis, there was an equal distribution with respect to ethnicity. However, they varied in the control source from clinic to the hospital, and in genotyping measurements from TaqMan to polymerase chain reaction-restriction fragment length polymorphism. Inconsistency with HWE was not seen in any control group (*P* > 0.10) [Table 1].

Meta-analysis results

As shown in Table 1, only one single study demonstrated that the -717GG genotype was associated with over two-fold greater risk of ischemic stroke compared to the -717AA genotype or the AA and GA genotypes (GG vs. AA, *OR* = 2.09, 95% *CI* = 1.01-4.35; GG vs. GA + AA, *OR* = 2.09, 95% *CI* = 1.01-4.33). Such a significant association was lost when all studies were pooled into a meta-analysis (GG vs. AA, *OR* = 1.12, 95% *CI* = 0.83-1.50, *P* = 0.207; GG + GA vs. AA, *OR* = 1.04, 95% *CI* = 0.93-1.17, *P* = 0.533; GG vs. GA + AA, *OR* = 1.10, 95% *CI* = 0.82-1.47, *P* = 0.220) [Figures 1 and 2, Table 1].

In terms of the analysis of -286C > T > A polymorphism in total subjects, we did not observe any statistical difference in each single dataset, nor in the combined dataset (AA vs. CC, *OR* = 0.86, 95% *CI* = 0.59 - 1.25, *P* = 0.348; AA vs. CC, *OR* = 0.92, 95% *CI* = 0.80 - 1.06, *P* = 0.609; AA vs. CC, *OR* = 0.89, 95% *CI* = 0.62 - 1.30, *P* = 0.374) [Figures 1 and 2, Table 1].

Table 1: Characteristics and summary ORs of studies included in the meta-analysis

Author	Year	Population	Cases/ controls	DNA source	Genetic variant	OR (95% CI) P_{Het}		
						Homozygote*	Dominant†	Recessive‡
Ladenvall	2006	Caucasian	599/600	Venous blood	-717A > G	1.00 (0.65, 1.56)	1.00 (0.82, 1.22)	1.00 (0.65, 1.54)
Ladenvall	2006	Caucasian	79/79	Venous blood	-286C > T > A	4.14 (0.46, 37.27)	1.12 (0.72, 1.73)	4.10 (0.46, 36.91)
Ben- Assayag	2007	Caucasian	217/520	Venous blood	-717A > G	1.33 (0.61, 2.92)	1.27 (0.93, 1.72)	1.20 (0.55, 2.60)
Wang	2009	Asian	564/564	Venous blood	-717A > G	2.09 (1.01, 4.35)	1.07 (0.84, 1.37)	2.09 (1.01, 4.33)
Wang	2009	Asian	31/30	Venous blood	-286C > T > A	0.76 (0.38, 1.52)	0.86 (0.67, 1.11)	0.81 (0.40, 1.60)
Shen	2013	Asian	546/994	Blood	-717A > G	0.77 (0.41, 1.46)	0.97 (0.78, 1.20)	0.77 (0.41, 1.46)
Shen	2013	Asian	542/994	Blood	-286C > T > A	0.82 (0.52, 1.31)	0.92 (0.76, 1.12)	0.86 (0.54, 1.36)
					-717A > G (total)	1.12 (0.83, 1.50)	0.207	1.04 (0.93, 1.17)
					-286C > T > A (total)	0.86 (0.59, 1.25)	0.348	0.92 (0.80, 1.06)
							0.609	0.89 (0.62, 1.30)
								0.220
								0.374

*GG vs. AA, AA vs. TT; †GG + GA vs. AA, AA + AT vs. TT; ‡GG vs. GA + AA, AA vs. AT + TT for -717A > G and -286T > A, respectively. OR: Odds ratio; CI: Confidence interval.

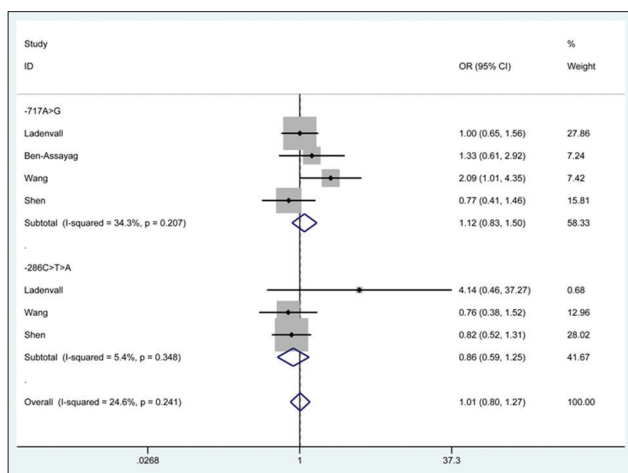


Figure 1: Overall estimates of C-reactive protein gene polymorphisms examined for ischemic stroke under the homozygote model. The summary odds ratio (OR) is shown by the middle of a solid diamond whose left and right extremes represent the corresponding 95% confidence interval. Horizontal axis represents ORs, which are calculated against controls for each study.

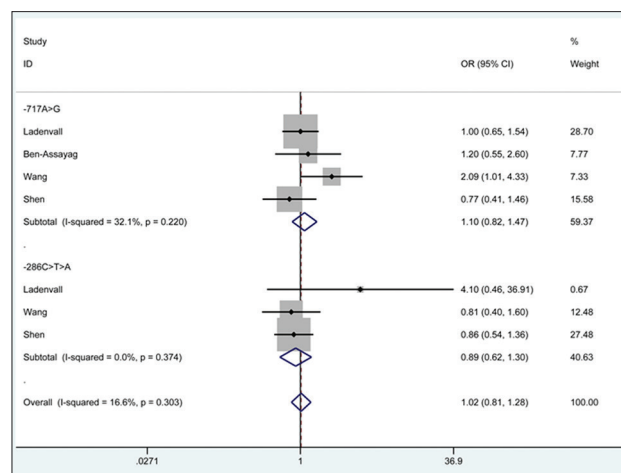


Figure 2: Overall estimates of C-reactive protein gene polymorphisms examined for ischemic stroke under the recessive model. The summary odds ratio (OR) is shown by the middle of a solid diamond whose left and right extremes represent the corresponding 95% confidence interval. Horizontal axis represents ORs, which are calculated against controls for each study.

Heterogeneity was not so obvious in studies of -717A > G polymorphism ($I^2 = 32.1\%$, $P = 0.220$) and there was no heterogeneity in the studies of the -286C > T > A polymorphism ($I^2 = 16.6\%$, $P = 0.303$).

Sensitivity analysis

We sequentially excluded one single study from the overall pooled analysis and recalculated the summary ORs to check whether the summary ORs were materially changed. The recalculated ORs were found stable, indicating our results were valuable (data not shown).

DISCUSSION

The identification of CRP gene as a marker of inflammation

degrees leads to great interest into the ischemic stroke genetic research.^[25] More and more epidemiologic and molecular evidence over the past 10 years has validated the CRP as an ischemic stroke susceptibility locus.^[26-30] Given that a minor change in the protein level of CRP, affected by the promoter polymorphisms, might lead to a significantly increased likelihood of ischemic stroke, lots of association researches have been performed to assess the possible relations between CRP -717A > G and -286C > T > A polymorphism and the ischemic stroke risk. However, these studies drew controversial conclusions. The majority of studies did not find these polymorphisms playing a role in the disease,^[16-18] but some study was on the other hand.^[15] Hence, we conducted this meta-analysis to assess the association between these two CRP polymorphisms and ischemic stroke risk.

Based on four studies, containing 4604 subjects for $-717A > G$ polymorphism and 1755 subjects for $-286C > T > A$ polymorphism, we found that the $-717A > G$ might not play a major role in the development of ischemic stroke, and the meta-analysis results showed that both of the C allele and the A allele of SNP $-286C > T > A$ were not associated with a significantly increased risk of ischemic stroke in general population. Our findings are consistent with the results of most included studies.

The most likely cause for our negative results is the inadequate sample size. Only four studies were included, and the small sample size could increase the probability of false negative. Moreover, complex interactions between genetic and environmental factors were reported to play important roles during the malignant progression of ischemic stroke. Ethnicity is one of the environmental factors. The incidence rate of ischemic stroke in Asians, especially in East Asian, is obviously lower than that of the Caucasians and blacks,^[31] suggesting that Asian populations may be less susceptible to ischemic stroke than Caucasian and black populations. Individuals of different ethnicities have different lifestyles, dietary patterns, and residential environments; other environmental factors include age, gender, family history and so on. An unfavorable combination of these factors may influence the results.

Although all the included studies were case-control studies, which had no inconsistency with HWE in any control group, no changed results of sensitive analysis, our meta-analysis still has some limitations. First, only four studies were included and the sample size is inadequate. Second, we are not allowed to identify whether the CRP gene polymorphisms predispose to ischemic stroke in an ethnicity-dependent manner or not, due to the inaccessibility of raw data. Third, genetic predisposition may differ depending on the age, gender, and other confounders. Hence interplay of genetic and environmental factors should be considered on condition that original articles report sufficient data. Moreover, as there may be unpublished data beyond our reach, we cannot assure inclusion of all studies which meet all of the selection criteria of this analysis, although we have put equal emphasis on publications during literature search. However, meta-analysis has stronger statistical power than any individual study in detecting genetic correlation, the results revealed in our analysis are trustworthy.

CONCLUSION

This meta-analysis revealed little evidence to support a role of CRP gene polymorphisms in ischemic stroke predisposition. However, to draw comprehensive and more reliable conclusions, further larger studies are needed to validate the association between CRP gene polymorphisms and ischemic stroke in various ethnic groups.

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