Original Article

C-reactive protein polymorphisms and genetic susceptibility to ischemic stroke and hemorrhagic stroke in the Chinese Han population

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Aim: The inflammatory marker C-reactive protein (CRP) has been strongly correlated with the risk of cardiovascular disease. Some single-nucleotide polymorphisms (SNPs) have been reported to be associated with serum CRP levels. In this study, we assessed the genetic association between SNPs within the *CRP* gene and ischemic and hemorrhagic stroke in the Han Chinese population.

Methods: This study comprises 564 ischemic stroke patients, 220 hemorrhagic stroke patients and 564 controls from the ethnic Han Chinese population in Wuhan. Four *CRP* SNPs, -757A>G (rs3093059), -717A>G (rs2794521), -286C>T>A (rs3091244) and +2147C>T (rs1205), were genotyped from patients using TaqMan assays.

Results: The A allele frequency for the -717A>G polymorphism was significant higher in controls than in ischemic stroke patients (P=0.037), after adjustment for traditional risk factors (odds ratio 0.28; 95% CI 0.12–0.65; P=0.003), suggesting a protective effect for this allele against ischemic stroke. Haplotype analysis showed that the H3 (G-C-C) haplotype conferred a significantly increased risk of ischemic stroke (odds ratio 1.052, 95% CI 1.001–1.106: P=0.047). Neither CRP genotypes nor haplotypes showed an association with hemorrhagic stroke. However, the frequency for haplotype H5 (A-T-C) was significantly higher in ischemic stroke than hemorrhagic stroke patients (P=0.0003).

Conclusions: These data suggest that the *CRP* gene -717A allele confers a protective effect against ischemic stroke. Furthermore, the H3 haplotype (G-C-C) is an independent risk marker for ischemic stroke, whereas the H5 haplotype (A-T-C) can be used as a prognostic marker of hemorrhagic stroke.

Keywords: C-reactive protein gene; stroke; genetic polymorphism
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Introduction

Stroke is a leading cause of death worldwide[1]. In China, the incidence of stroke is much higher than that of coronary heart disease compared with incidences recorded for Western countries[2]. Among all subtypes, ischemic stroke accounts for the majority of cases in the Chinese population, about 60%, and cerebral hemorrhage accounts for another 20%–40%[3]. In contrast, the overall incidence of stroke and the proportion of hemorrhagic strokes are lower in most Western countries than in China[3–6]. These different epidemiological patterns may be due to differences in genetic backgrounds of the populations.

Stroke is a heterogeneous multifactorial disorder. Acute cardiovascular events and stroke are thought to be caused largely by inflammation-mediated destabilization and rupture of atherosclerotic lesions[7, 8]. Numerous classic risk factors such as hypertension, diabetes mellitus, hyperlipidimia and smoking contribute to this disease[9–12], but family and twin-based studies suggest the involvement of genetic factors in the development of stroke[13–15]. By case-controlled association studies, several inflammatory molecular genotypes

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have been suggested to be associated with increased stroke risk[16].

C-reactive protein (CRP), an acute inflammatory phase reactant, is a marker of systemic inflammation[17]. It has also evolved as a novel plasma marker for atherothrombotic disease and may reflect the level of inflammatory activity within atherosclerotic plaques[18–20]. Therefore, CRP serum level is a valuable and sensitive indicator of initial and recurrent cerebrovascular events[21–23]. Genetic variants in the *CRP* gene may more accurately reflect lifetime CRP exposure than serum CRP level measured at a single time point. Understanding of *CRP* genetic variation in stroke risk has been limited and controversial, especially for hemorrhagic strokes. Recently, a case-controlled study of a Japanese population suggested a strong association between CRP 1059G>C (rs1800947) polymorphism and ischemic stroke[24]. On the contrary, no association was detected between this allele and ischemic stroke risk in a Swedish population[25]. To address the role of CRP variability in stroke risk, we genotyped four CRP SNPs in the Han Chinese population. Our data support the association between CRP genetic variants and the risk of both ischemic and hemorrhagic stroke.

Materials and methods

Study population and data collection This multicenter study for the assessment of risk factors for stroke was sponsored by the Ministry of Science and Technology of China. The study protocol was approved by the review boards of the Ministry of Public Health, the Ministry of Science and Technology of China, and the ethics committees at all participating hospitals. Informed consent was obtained from all participants.

The study population was composed of 564 patients with ischemic stroke, 220 patients with primary cerebral hemorrhage, and 564 control subjects. All patients and control subjects were recruited from patients consecutively admitted to five hospitals in Wuhan, China, from November 2004 to June 2006. Diagnosis of stroke was based on medical history, neurological examination, and CT or MRI according to the International Classification of Diseases, ninth edition. Only patients with one of these three subtypes of stroke were included: cerebral thrombosis (atherothrombosis), lacunar infarction (lacunar), and intracerebral hemorrhage. Other types of stroke (including subarachnoid hemorrhage, cardiogenic embolic brain infarction, brain tumors and cerebrovascular malformations), severe systemic diseases (except diabetes mellitus), severe inflammatory diseases, autoimmune disease, tumors, and serious chronic diseases (*eg*, hepatic cirrhosis, renal failure) were excluded. Controls were randomly selected from inpatients with minor illnesses from the Departments of Ophthalmology, Gastroenterology, Otorhinolaryngology and Orthopedics, or were healthy individuals from the community. The exclusion criteria for control subjects were the same as those in the aforementioned patient group. Controls had no relationship with the stroke cases and no family history of stroke. All subjects were unrelated Han Chinese individuals originating from the same geographical area.

Data collection and definition of risk factors Information on demographics and other risk factors was collected by use of a structured questionnaire involving the history of hypertension, diabetes, hyperlipidemia, smoking, and physical exercise, including evaluation of body mass index.

Hypertension was defined as a systolic blood pressure ≥140 mmHg, a diastolic blood pressure ≥90 mmHg, or current treatment with an antihypertensive drug. Diabetes was diagnosed by a fasting glucose level of >7.8 mmol/L, a glucose level of >11.1 mmol/L at 2 h after oral glucose challenge, or both. Hyperlipidemia was defined as total plasma cholesterol level of >5.72 mmol/L or plasma triglyceride >1.70 mmol/L. If a subject had smoked at least 1 cigarette per day for at least 1 year and had not quit smoking by the time of the study, he/she was defined a current smoker.

Blood sample collection, biochemical variables and genomic DNA extraction Blood was drawn from an arm vein into a sterile tube containing ethylenediamine tetraacetic acid (EDTA). Plasma samples were obtained from fasting participants and stored at -80 °C. Plasma biochemical parameters were assayed using an automatic analyzer (7060, Hitachi). Seven hundred and sixty-one subjects, including 431 subjects of ischemic strokes, 67 subjects of hemorrhagic strokes and 263 controls, were randomly selected for CRP level measurement. CRP level was measured with a Dade Behring BNII nephelometer. Genomic DNA was extracted using the QG-Mini80 workflow with DB-S kit (FUJIFILM Corporation, Tokyo, Japan) per the manufacturer's instructions. DNA was diluted to a final concentration of 10 ng/μL and stored at -80 °C for genotyping.

TagSNP selection and genotyping Based on information in the NCBI SNP database and International HapMap project data (http://www.hapmap.org) for the Han Chinese population, SNPs with a minor allele frequency >10% and previously published SNPs were selected for the study[26, 27], including -757A>G (rs3093059), -717A>G (rs2794521), and -286C>T>A (rs3091244) located in the promoter region and +2147C>T (rs1205) in the 3′ flanking region.

TagSNPs were genotyped using the TaqMan SNP Geno-

typing Assay. The three diallelic SNPs were designed and supplied commercially by GeneCore Inc (GeneCore, Shanghai). The triallelic variant was supplied by Applied Biosystems Inc (ABI, Foster City, CA). The primer and probe sequences of these SNPs are listed in Table 1. Each probe was dually labeled with a reporter dye at the 5′ end and contained a minor groove-binding group (MGB) at the 3′ end. The polymerase chain reactions for tagSNPs (-757A>G, -717A>G, +2147C>T) were carried out in 5 μL final volume and were composed of 1×Universal Mastermix (ABI), two TaqMan probes at 0.2 μmol/L each, two primers at 1 μmol/L each and 1 ng/μL genomic DNA. Thermal cycling reactions were performed as follows: 50 °C for 2 min, 95 °C for 10 min, and then 50 cycles at 95 °C for 15 s and 60 °C for 1 min. Amplifications were carried out on the ABI 7900HT instrument. Allelic discrimination was measured automatically using Sequence Detection Systems 2.1 software (autocaller confidence level 95%). Primers, TaqMan probes and PCR conditions used for genotyping and analysis of triallelic SNP (rs3091244) have been described previously by Carlson *et al*[28]. A total of 10% of samples were re-genotyped for each SNP at random, and all results were consistent. In addition, all DNA samples for cases and controls were blindly run in the same batches.

Statistical analysis Statistical analyses were performed with the SPSS 13.0 software package (SPSS Inc, Chicago) for Windows (Microsoft Corp, Redmond, Wash) and haplo.stats version 1.2.1 for the R programming language. Summary statistics were expressed as means±standard error (SEM) or as percentages, and CRP levels were expressed as medians. The χ^2 test was used to assess the deviation from Hardy-Weinberg equilibrium for genotype frequencies and to examine genotype and allele frequencies between cases and controls. Frequencies of categorical variables were compared by χ^2 test or Fisher's exact test. Continuous variables were compared between cases of stroke and controls using Student's *t*-test. CRP levels had a skewed distribution and nonparametric tests were used. The potential independent role of each SNP on stroke was investigated with multiple logistic regression analyses adjusted for age, sex, BMI, hypertension, hyperlipidemia, diabetes mellitus and smoking status. To avoid spurious allelic associations, a probability value <0.0125 (0.05/4) was considered statistically significant after *Bonferroni* correction was applied to conservatively identify the susceptibility variants on CRP. The association between CRP levels and CRP genotype was investigated with stepwise linear regression adjusted for the above-mentioned risk factors. CRP levels were natural log transformed in this model. The r^2 measurement was used to determine linkage disequilibrium (LD) with the software EMLD (http://request.mdacc.tmc.edu/qhuang/Software/pub.htm). The frequency distribution of haplotypes was calculated by the haplo.stats software. Haplotype-based hypothesis tests of generalized linear models were conducted using haplo.stats, in which ischemic or hemorrhagic stroke status was the dependent variable, and haplotypes and the other classic risk factors were independent and covariate variables[29].

The present study had 80% power to detect an association with OR <0.40 (assuming a protective effect) for alleles ≥15% frequency assuming a two-sided level at 0.05 using the QUANTO program in recessive model[30]. $P<0.05$ was considered statistically significant, and all statistical tests were two sided.

Results

Characteristics of patients and control subjects The baseline characteristics of all participants are summarized in Table 2. The mean age was older in the control subjects than in either ischemic or hemorrhagic stroke patients. As expected, the frequencies of hypertension, serum CRP levels, systolic blood pressure and diastolic blood pressure were

Table 1. Sequences of TaqMan primers and probes.

	Primer (5'→3')	Probe
rs3093059	Forward TTTGGTTTTTGCATGGACACA	FAM-CTCAGCCAATTGAGTACA-MGB
	Reverse TGTCAGGGCCGTCATTTAGTG	TET-TCTCAGCCGATTGAGTA-MGB
rs2794521	Forward CCGTCATTTAGTGCCAAGATGTC	FAM-ACCGCATGTTCTC-MGB
	Reverse CCTTCCTGTGTCCAAGTATTCTCAT	TET-CACCGCGTGTTCT-MGB
rs1205	Forward GGCTCCTCCACTTCCAGTTTG	FAM-CTGTCCTCACAGTCT-MGB
	Reverse ACCACCAGTAGCCATCTTGTTTG	TET-TGTCCTCATAGTCTCT-MGB
rs3091244	Forward TGTTGGAGAGGCAGCTACCA	NED-ATGGCCACTAGTTTA-MGB
	Reverse TCCTGCGAAAATAATGGGAAA	VIC-ATGGCCACTTGTTTA-MGB
		FAM-TGGCCACTCGTTTAA-MGB

Table 2. Characteristics of the study population.

	Control	Case			
	(*n*=564)	Ischemic stroke		Hemorrhagic stroke	
		(*n*=564)	*P* value	(*n*=220)	*P* value
Age (years)	62.23±0.39	61.01±0.42	0.032[b]	57.58±0.75	<0.001[b]
Male (%)	62.6	64.5	0.496	67.3	0.220
BMI	23.67±0.14	24.46±0.15	<0.001[b]	23.75±0.23	0.759
Systolic BP (mmHg)	131.03±0.87	146.53±0.99	<0.001[b]	153.67±1.71	<0.001[b]
Diastolic BP (mmHg)	78.69±0.47	86.44±0.59	<0.001[b]	93.57±1.14	<0.001[b]
Total cholesterol (mmol/L)	4.07±0.09	4.54±0.05	0.176	4.34±0.72	0.423
Hypertension, *n* (%)	103 (18.3)	391 (69.3)	<0.001[b]	144 (65.5)	<0.001[b]
Hyperlipidemia, *n* (%)	118 (20.9)	198 (35.1)	<0.001[b]	26 (11.8)	0.003[b]
Diabetes mellitus, *n* (%)	19 (3.3)	101 (17.9)	<0.001[b]	9 (4.1)	0.624
Smoking (current), *n* (%)	141 (25.0)	153 (27.1)	0.416	68 (30.9)	0.093
CRP, mg/L Median (IQ-range)	0.61 (0.32–1.46)	1.79 (0.70–4.87)	<0.001[b]	6.58 (1.42–19.41)	<0.001[b]

BMI=body mass index. [b]*P*<0.05 *vs* controls.

higher in stroke patients than in controls. The proportions of sex, smoking status, and total cholesterol level did not differ between stroke patients and controls. Body mass index and frequencies of diabetes mellitus and hyperlipidemia were higher in ischemic stroke patients than in controls; however, the frequency of hyperlipidemia was lower in hemorrhagic stroke patients. Body mass index and the frequency of diabetes mellitus did not differ between hemorrhagic stroke patients and controls.

CRP genotypes in relation to ischemic stroke, hemorrhagic stroke and CRP level All genotype distributions were consistent with the Hardy-Weinberg equilibrium except -757A>G, which was removed from further analysis. Genotype frequencies are shown in Table 3. No significant differences were observed in genotype distributions between hemorrhage patients and controls. However, the -717A>G genotype frequencies in the recessive model (AA+AG *vs* GG) differed significantly between ischemic stroke patients and controls (*P*=0.037). Furthermore, after adjustment for age, gender, BMI, and frequencies of hypertension, diabetes mellitus, hyperlipidemia and smoking, -717A>G was still associated with ischemic stroke (AA+AG *vs* GG, odds ratio 0.28; 95% CI 0.12–0.65; *P*=0.003, Table 4). Furthermore, this SNP was still significantly associated with ischemic stroke after a *Bonferroni* adjustment. Age (odds ratio 0.96; 95% CI 0.94–0.97; *P*<0.001), hypertension (odds ratio 11.46; 95% CI 8.36–15.71; *P*<0.001), and diabetes mellitus (odds ratio 3.77; 95% CI 2.11–6.73; *P*<0.001) were also estimated as independent predictors of ischemic stroke (Table 4). Nevertheless, there was no association between any genotype and serum CRP levels after adjustment for risk factors (Table 5).

Haplotype analysis Pairwise LD measures and correlation coefficients between CRP polymorphisms were analyzed. None of our tagSNPs were high in LD (r^2≤0.30). We used haplo.stats software to calculate haplotypes based on the observed genotypes. To minimize potential false positive associations conducted in haplotype analysis, haplotypes were not considered if all estimated frequencies are less than 5% in controls and cases. No significant association was detected for any haploytpe and ischemic stroke.

Next, we analyzed the independent effect of haplotype on ischemic stroke after adjustment for other risks. Haplotype H1 was the most frequent and was thus chosen as the baseline. As expected, haplotype H3 was associated with an increased risk of ischemic stroke (odds ratio 1.052, 95% CI 1.001–1.106: *P*=0.047; Table 6). Other risk factors that showed significant effects on ischemic stroke were also listed in Table 6. These results were consistent with logistic regression analysis. Haplotype H5 was significantly less frequent in hemorrhagic stroke patients than in controls (2.7% versus 5.5%, *P*=0.024; Table 7). In contrast, haplotype H3 was higher in hemorrhagic stroke patients, with marginal significance (14.7% versus 18.6%, *P*=0.054; Table 7). However, after the adjustment of conventional risk factors, haplotypes H3 and H5 failed to show a significant difference between controls and hemorrhagic stroke patients (*P*=0.075 and *P*=0.064, respectively; data not shown).

Difference in allele frequencies and haplotype between ischemic stroke and hemorrhagic stroke The allele frequencies did not differ significantly between ischemic stroke or hemorrhagic stroke and controls. However,

Table 3. Genotype distribution in patients with ischemic and hemorrhagic stroke and control subjects.

SNP	Genotype	Controls (n=564)	Ischemic stroke (n=564)			Hemorrhagic stroke (n=220)		
		n	n	Crude P value	Adjusted P value	n	Crude P value	Adjusted P value
rs2794521	Additive							
	AA	398	386	0.110	0.009[b]	143	0.247	0.164
	AG	155	155			70		
	GG	11	23			7		
	Recessive							
	AA+AG	553	541	0.037[b]	0.003[b]	213	0.301	0.142
	GG	11	23			7		
	Dominant							
	AA	398	386	0.438	0.202	143	0.130	0.135
	GG+AG	166	178			77		
rs3091244	Additive							
	CC	323	340	0.125	0.262	137	0.358	0.357
	CA	157	134			57		
	CT	53	60			12		
	AA	19	15			11		
	TT	5	3			1		
	AT	7	12			2		
rs1205	Additive							
	TT	173	172	0.440	0.194	68	0.686	0.109
	TC	297	282			110		
	CC	94	110			42		
	Recessive							
	TT+TC	470	454	0.216	0.085	178	0.421	0.056
	CC	94	110			42		
	Dominant							
	TT	173	172	1.000	0.999	68	0.949	0.793
	TC+CC	391	392			152		

Crude P values were determined by a 95% two-sided χ^2 test. Adjusted P value was calculated by multiple logistic regression analysis with adjustment for age, sex, BMI, history of hypertension, hyperlipidemia, diabetes mellitus and smoking. [b]P<0.05 *vs* controls.

Table 4. Multivariate logistic regression analysis of determinants of ischemic stroke.

Variable	Adjusted OR	95% CI	P value
Age (year)	0.96	0.94–0.97	<0.001[b]
Sex (M/F)	0.96	0.70–1.33	0.824
BMI	1.00	0.96–1.04	0.966
Hypertension(y/n)	11.46	8.36–15.71	<0.001[b]
Diabetes mellitus(y/n)	3.77	2.11–6.73	<0.001[b]
Hyperlipidemia (y/n)	1.35	0.97–1.89	0.077
Smoking (y/n)	0.81	0.57–1.16	0.253
rs2794521, AA+AG *vs* GG	0.28	0.12–0.65	0.003[b]

Variables included in the model were sex, age, BMI, hypertension, hyperlipidemia, diabetes mellitus, and smoking. [b]P<0.05 *vs* controls.

the frequency for -286T was significantly higher in ischemic than in hemorrhagic stroke patients, whereas -286A was higher in hemorrhagic stroke patients (P=0.027, Table 8). In addition, the frequency for haplotype H5 (A-T-C) was significantly higher in ischemic stroke than hemorrhagic stroke (6.9% *vs* 2.7%, P=0.0003, data not shown).

Discussion

To our knowledge, this is the first study assessing the contribution of the *CRP* gene to ischemic and hemorrhagic stroke in the Han Chinese population. We have shown here that the -717A>G polymorphism is not only associated with ischemic stroke, but also an independent predictor of ischemic stroke in this ethnic group.

Table 5. Association of CRP genotype with plasma CPR level.

SNP	Genotype	CRP, mg/L Median (IQ-range)	Adjusted *P* value
rs2794521	AA	1.300 (0.464–3.630)	0.280
	AG	1.345 (0.525–4.122)	
	GG	1.362 (0.406–4.660)	
rs30912244	AA	2.925 (0.656–5.733)	0.220
	AC	1.351 (0.530–4.595)	
	AT	2.650 (1.148–7.983)	
	CC	1.270 (0.422–3.400)	
	CT	1.420 (0.550–4.359)	
	TT	0.400 (0.230–3.477)	
rs1205	CC	1.420 (0.629–4.840)	0.444
	CT	1.318 (0.490–3.638)	
	TT	1.095 (0.390–3.370)	

Adjusted *P* value was calculated by stepwise linear regression analysis with adjustment for age, sex, BMI, history of hypertension, hyperlipidemia, diabetes mellitus and smoking.

Table 6. Association between haplotype and ischemic stroke.

	Coefficient	Odds ratio	95% CI	*P* value
Age	–0.007	0.993	0.990–0.995	8.9×10^{-16} [b]
Sex	0.003	1.003	0.948–1.061	0.917
BMI	–0.000	1.000	0.993–1.007	0.946
Hypertension	0.507	1.660	1.575–1.750	0.000[b]
Diabetes mellitus	0.184	1.202	1.106–0.977	1.7×10^{-5} [b]
Hyperlipidemia	0.049	1.050	0.991–1.112	0.097
Smoking	0.032	1.032	0.970–1.099	0.318
H2 (A-A-C)	–0.008	0.991	0.943–1.043	0.742
H3 (G-C-C)	0.051	1.052	1.001–1.106	0.047[b]
H4 (A-C-C)	–0.015	0.985	0.915–1.061	0.692
H5 (A-T-C)	0.006	1.006	0.935–1.082	0.873
H1 (A-C-T)		1.000		

Variables included in the model were sex, age, BMI, hypertension, hyperlipidemia, diabetes mellitus, and smoking. The H1 (A-C-T) haplotype was the reference group. [b]*P*<0.05 *vs* controls.

Table 8. Allele frequency between ischemic stroke and hemorrhagic stroke.

SNP	Alleles	Ischemic stroke/%	Hemorrhagic stroke/%	*P* value
rs2794521	A	0.822	0.809	0.567
	G	0.178	0.191	
rs3091244	A	0.156	0.184	0.027[b]
	C	0.775	0.780	
	T	0.069	0.036	
rs1205	C	0.445	0.441	0.883
	T	0.555	0.559	

[b]*P*<0.05 ischemic stroke group *vs* hemorrhagic stroke group.

-717A>G is located in the promoter region of the *CRP* gene. Our results suggested that -717A was a protective allele against ischemic stroke. Previous studies have indicated that the -717A allele is associated with increased risk for coronary heart disease and diabetes[27, 31], although contradictory results have also been reported[32]. Because stroke and coronary heart disease are both considered atherosclerosis-related diseases, they share a variety of common pathogenic mechanisms. This discrepancy could be due to a number of factors. First, -717A>G could be involved in the development of these two diseases by different mechanisms. The transition of -717A>G creates a potential binding site for glucocorticoid receptor (GR), which may result in changes in *CRP* expression. Consistent with this model, previous studies demonstrated that the -717G allele was associated with elevated CRP concentrations during the acute phase of stroke or transient ischemic attack[33]. Alternatively, population stratification caused by inappropriate sampling could be an influence. However, the allelic frequencies for the -717A>G polymorphism in ischemic stroke patients were actually quite similar to the previously published data[33], suggesting that the possibility of a false association was low. Finally, false positive results due to genotyping errors are

Table 7. Haplotype frequency estimates.

Haplotype	Allele -717	Allele -286	Allele 2147	Control Frequency	Ischemic stroke Frequency	Ischemic stroke *P*	Hemorrhagic stroke Frequency	Hemorrhagic stroke *P*
H1	A	C	T	0.551	0.542	0.704	0.542	0.772
H2	A	A	C	0.165	0.149	0.250	0.178	0.545
H3	G	C	C	0.147	0.170	0.131	0.186	0.054
H4	A	C	C	0.058	0.057	0.876	0.048	0.492
H5	A	T	C	0.055	0.069	0.210	0.027	0.024[b]

Haplotypes were not listed if all the estimated frequencies were less than 5% in controls and stroke cases. [b]*P*<0.05 *vs* controls.

also very unlikely. In our study, we used the TaqMan SNP Genotyping System, which has much higher specificity and accuracy than previously used approaches. We randomly re-genotyped about 10% of the samples and failed to identify any discrepancies in our results.

Haplotype analysis also identified a significant association between haploytpe H3 and ischemic stroke. Compared with haplotype H1, haplotype H3 was an independent risk predictor of ischemic stroke. These two haplotypes differed only by the -717G and +2147C alleles. Previous studies have indicated that -717G and +2147C alleles are associated with high CRP levels[33, 34]. Therefore, this may explain why the H3 haplotype is associated with increased risk of ischemic stroke. In addition, the haplotype containing the -717G allele showed this effect on ischemic stroke, consistent with our logistic regression analysis. In hemorrhagic stroke patients, the frequency of haplotype H5 was lower than that of controls. However, H5 was not an independent protective factor against hemorrhagic stroke, and previous studies indicated that the -286T and +2147C alleles were also associated with high CRP levels[25, 28, 34, 35]. One possible explanation for this finding is that the SNPs of -286C>T>A and +2147C>T are markers of other confounding factors associated with low risk of hemorrhagic stroke.

Another interesting finding in our study is the significant difference in the triallelic SNP (-286C>T>A) frequency between ischemic and hemorrhagic stroke patients. This difference was mainly displayed in the distribution of minor alleles such as -286A or -286T. The ischemic group contained more -286T and less -286A, but the hemorrhage group was the opposite, with more -286A and less -286T. Recent functional analysis studies implicate the triallelic -286C>T>A promoter SNP as a functional variant, as the upstream stimulatory factor-1 can bind to it when the -286T is present[28, 36]. Consistent with this, previous studies demonstrated that the -286T and -286A alleles were associated with higher plasma CRP levels[25, 34]. To our knowledge, this is the first report showing genetic determinants for the distinction between ischemic and hemorrhagic stroke risk. However, because of the functional complexity in -286C>T>A SNP, the significance of differences in this allele frequency for the ischemic stroke and hemorrhagic stroke remains to be elucidated.

Our analysis failed to show an association between any SNP and serum CRP level in our study population, which is inconsistent with previous reports and our observations between genotyping and strokes. This may be due to a number of factors. On one hand, serum CRP levels can be affected by many conditions including infection, inflammatory events, smoking, diet and medications such as statins. These factors may disguise the association between serum CRP level and genotype. On the other hand, a previous report showed the association between -717A>G polymorphism and CRP levels only during acute stroke[33], and some of our subjects blood samples were taken during the non-acute phase of the stroke.

In conclusion, potential associations between *CRP* gene polymorphisms and stroke risk were investigated in a Han Chinese population. We found that the -717A allele was significantly associated with lower risk for ischemic stroke and haplotype H3 (G-C-C) was an independent risk of ischemic stroke. No significant differences were observed in genotype distributions between hemorrhagic patients and controls. However, haplotype H5 (A-T-C) can be used as prognostic markers of hemorrhagic stroke.

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Author contribution

Qi WANG, Hu DING, and Dao-wen WANG designed this research; Qi WANG, Hu DING, Jia-rong TANG, Lan ZHANG, Yu-jun XU, and Jiang-tao YAN performed the research; Hu DING, Wei WANG, Ru-tai HUI, and Cong-yi WANG contributed new analytical tools and reagents; Qi WANG and Hu DING analyzed data; Qi WANG and Dao-wen WANG wrote the paper.

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