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Original Article

Association between *CRP* polymorphisms and the risk of ischemic stroke

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Abstract: Purpose: This study aimed to detect the potential association between the single nucleotide polymorphisms (SNPs) of rs1130864, rs3093059 and rs1205 in C-reactive protein (*CRP*) gene and the risk of ischemic stroke (IS). Methods: 100 diagnosed IS patients and 106 healthy people were recruited into the case and control groups respectively in this study. The genotypes distributions of the control groups in rs1130864, rs3093059 and rs1205 were detected by Hardy-Weinberg equilibrium (HWE). TaqMan fluorescence probe was adopted to conduct quantitative detection and high throughput fluorescent quantitation PCR instrument was used to distinguish genotypes. Haploview software was applied to perform linkage disequilibrium and haplotype analysis. Odds ratio (OR) with 95% confidence interval (CI) was calculated by chi-square test to assess the association of *CRP* SNPs and haplotypes with IS risk. Results: The genotypes frequencies of rs1130864, rs3093059 and rs1205 in control group were all consistent with HWE. No statistically significant difference was found in genotypes and alleles distributions of rs1130864, rs3093059 and rs1205 in *CRP* gene between two groups ($P>0.05$). The haplotype C-C-C could significantly increase the risk of IS (OR=1.74, 95% CI=1.04-2.93). Conclusion: The rs1130864, rs3093059 and rs1205 polymorphisms of *CRP* gene are not related to the occurrence of IS, while C-C-C haplotype may be a risk factor for IS.

Keywords: Ischemic stroke, c-reactive protein, single nucleotide polymorphisms

Introduction

Ischemic stroke (IS), also called cerebral infarction, is a kind of neurologic impairment syndrome caused by local disturbance of brain blood circulation. Its development is often accompanied by relative neurological diseases: hemiplegia, sensory nerves disorder, aphasia, hemianopsia etc. [1, 2]. The disease is featured by high morbidity, disability and mortality, which seriously influences human health and life quality. Thrombolytic therapy is an effective method to relieve the symptoms of patients who are treated between 3 and 6 h after stroke, but this method may cause patients hemorrhage [3, 4]. Since this multifactorial disease is affected by genetic and environmental factor, it is an effective and direct cure way to find and modify relative genes with IS. Genetic polymorphisms attract more attention on exploring the pathogenesis of IS.

C-reactive protein (*CRP*) gene, located in chromosome 1q23 [5]. The CRP proteins are relevant to signal transmission between cells and immune system. Also, they can activate alexin to promote phagocytosis and exert the effects on immune regulation [6]. Besides, CRP is regarded as a marker of inflammation and tissue damage [7, 8]. Recently, a number of studies have confirmed that *CRP* participates in the occurrence and development of myocardial infarction, coronary heart disease and IS [9-12]. Furthermore, the concentration of *CRP* could be regulated by certain polymorphisms [13, 14].

So we chose 3 SNPs of rs1130864, rs3093059 and rs1205 in *CRP* gene to analyze the association of these polymorphisms with IS susceptibility so as to supply more theoretical evidence for the early diagnosis and treatment of IS.

Materials and methods

Subjects

100 IS patients hospitalized in The Affiliated Hospital of Luzhou Medical College from June 2011 to September 2013 were enrolled in the case group and 106 healthy people from physical examination center of the same hospital

Table 1. The detailed information of SNPs in CRP

SNPs	Location	Allele (major/minor)
Rs1130864	3'UTR	C/T
Rs3093059	Promoter	T/C
Rs1205	3'UTR	C/T

Table 2. Comparison of clinical data

Clinical feature	Controls	Cases	*P*
Age (year)	62.19±2.89	63.25±3.15	>0.05
Gender (male/female)	54/52	51/49	>0.05
Diastolic blood pressure	81.46±9.59	89.17±10.65	<0.05
Systolic blood pressure	135.34±18.21	146.29±19.35	<0.05
GLU (mmol/L)	5.84±1.64	6.25±1.56	>0.05
TG (mmol/L)	1.53±0.78	1.55±1.18	>0.05
TC (mmol/L)	5.26±0.64	4.29±0.75	<0.05
LDL-C (mmol/L)	2.18±0.49	2.70±0.55	<0.05
HDL-C (mmol/L)	1.18±0.22	1.20±0.21	>0.05
II diabetes (%)	11.0 (11.00)	23.0 (23.00)	<0.05
Hypertension (%)	38 (38.00)	59 (59.00)	<0.05
Hs-CRP (mg/L)	1.857±1.035	3.462±1.157	<0.05

Note: GLU: blood glucose; TG: triacylglycerol; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein.

during the same period were gathered as the controls. All patients were confirmed though the examinations of CT and MRI which excluded the possibility of IS caused by external factors such as trauma. They were unrelated by blood and experienced no radiotherapy or chemotherapy before blood collection. The controls were chosen randomly matched with the patients by gender, residence, resident year and their age difference was less than 5 years. As for age, gender, nationality, native place and other basic information, there was no apparent difference between two groups (P>0.05). The patients were aged 53~74 with an average age of 63.25±3.15, while the controls were aged from 55 to 75 with the average age of 62.19±2.89. This study obtained the approval from the Ethics Committee of the hospital and written informed consents were obtained from all participants or their families.

Methods

Collection of clinical data

Epidemiology information of all participants, including age, gender, histories of hypertension and II diabetes were collected with questionnaire method. Meanwhile, the blood pressures were recorded and the blood samples were collected to test blood glucose (GLU), triacylglycerol (TG), total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C) and the level of plasma hs-CRP.

SNPs selection

The SNPs of *CRP* were chosen using the online human genomic haplotype database (http://hapmap.ncbi.nlm.nih.gov) with the following inclusion criteria: MAF≥0.05, Han Chinese, and the data from HapMap Data Rel 24/phase II Nov08, on NCBI B36 assembly dbSNP b126. With considering the polymorphisms of *CRP* that were closely related to IS in previous reports, three polymorphisms including rs1130864, rs3093059 and rs1205 were adopted in this study. The detailed SNPs information were shown in **Table 1**.

DNA extraction

4 mL peripheral venous blood was collected from every subject and put in EDTA anticoagulation tubes. Blood of IS patients was obtained after 72 h when they were confirmed by physicians. Genome DNA from all subjects were extracted using conventional phenol-chloroform method and stored at -20°C refrigerator.

Genotyping

TaqMan fluorescence probe was employed to conduct quantitative detection. The probes and primers were designed and synthezed from American Applied Biosystems company. PCR reaction was performed in a total volume of 25 uL, containing 1.25 μL probe/primer Mix, 1.0 μL template DNA and 12.5 μL TaqMan Universal PCR Master Mix and 10.25 μL redistilled water. PCR conditions were 50°C for 2 min, 95°C for 10 min, with 45 cycles containing 90°C for 20 s and 58°C for 50 s. The PCR products were preserved at 4°C. The genotypes were analyzed by ABI7900 high throughput fluorescent quantitative PCR instrument (American Applied Biosystems company).

Table 3. The comparison of genetype and allele frequencies between groups

SNPs	Genotype/Allele	Case, n (%)	Control, n (%)	OR (95% CI)	*P*
Rs1205	CC	49 (49.00)	47 (44.34)	1.00 (Ref.)	-
	CT	38 (38.00)	40 (37.74)	0.91 (0.50-1.66)	0.76
	TT	13 (13.00)	19 (17.92)	0.66 (0.29-1.48)	0.31
	C	136 (68.00)	134 (63.21)	1.00 (Ref.)	-
	T	64 (32.00)	78 (36.79)	0.81 (0.54-1.22)	0.31
Rs1130864	CC	69 (69.00)	74 (69.81)	1.00 (Ref.)	-
	CT	27 (27.00)	30 (28.30)	0.97 (0.52-1.79)	0.91
	TT	4 (4.00)	2 (1.89)	2.15 (0.38-12.08)	0.38
	C	165 (82.50)	178 (83.96)	1.00 (Ref.)	-
	T	35 (17.50)	34 (16.04)	1.11 (0.66-1.86)	0.69
Rs3093059	TT	52 (52.00)	67 (63.21)	1.00 (Ref.)	-
	TC	39 (39.00)	34 (32.08)	1.48 (0.82-2.65)	0.19
	CC	9 (9.00)	5 (4.71)	2.32 (0.73-7.34)	0.14
	T	143 (71.50)	168 (79.25)	1.00 (Ref.)	-
	C	57 (28.50)	44 (20.75)	1.52 (0.97-2.39)	0.07

Note: a, the major allele; b, the minor allele.

Table 4. The haplotype analysis of CRP polymorphisms

Haplotypes 1-2-3	Cases, 2 n	Controls, 2 n	OR (95% CI)	*P*
T-C-T	58	78	1.00 (Ref.)	-
C-C-C	57	44	1.74 (1.04-2.93)	0.04
C-T-T	35	34	1.38 (0.77-2.48)	0.27
C-C-T	42	46	1.23 (0.72-2.11)	0.46

Note: 1-2-3 indicates rs1205, rs1130864, rs3093059, respectively.

Statistics analysis

All measurement data were represented with $\bar{x}$ ±s and the linkage disequilibrium in polymorphisms was analyzed with Haploview 4.2 software. Odds ratio (OR) with 95% confidence interval (CI) was calculated by chi-squared test to compare the frequency differences of genotypes, alleles and haplotypes between the case and control groups. The genotype distributions of the control groups in rs1130864, rs3093059 and rs1205 were tested by Hardy-Weinberg equilibrium (HWE). $P<0.05$ indicates statistical significance. All the analyses were completed in SPSS18.0 software.

Results

Comparison of clinical data

The proportions of individuals with II diabetes and hypertension histories and the level of hs-CRP were higher in the case group compared with controls ($P<0.05$). The cases also displayed significantly higher incidence rate of conventional vascular risk factors ($P<0.05$), including high blood pressure, LDL-C and TC levels. As for age, gender, GLU, TG and HDL-C, there were no statistically significant differences between two groups ($P>0.05$). The results were shown in **Table 2**.

Comparison of genotypes and alleles frequencies of SNPs

The genotypes distributions and alleles frequencies of 3 SNPs (rs1130864, rs3093059 and rs1205) in *CRP* suggested that there were no statistical differences between the cases and controls ($P>0.05$, **Table 3**).

Haplotype analysis of CRP polymorphisms

The linkage disequilibrium analysis was conducted. If the haplotype frequency was less than 5%, it was rejected. So four haplotypes were studied to evaluate their relevances with IS risk (**Table 4**). The outcome suggested that haplotype C-C-C was associated with the increased risk of IS (OR=1.74, 95% CI=1.04-2.93).

Discussion

Cerebrovascular disease is the leading cause of death in China, especially stroke of ischemic

and hemorrhagic strokes (HS). IS accounts for 70% of cerebrovascular cases [3]. More and more studies devote to exploring the pathogenesis and therapies of IS. Genetic variant becomes a hot topic. In the study of Zhang et al., rs11053646 polymorphism of *OLR-1* gene was confirmed as a marker for the IS risk and prognosis in Chinese population [15]. In Turkish population, the genotype and allele frequencies of *ACE* polymorphisms showed no significant differences between IS patients and healthy controls [16], although it has been reported that this polymorphism is associated with an increased risk of stroke in other populations [17, 18]. A meta-analysis conducted by Kelly et al. suggested that TT genotype of *MTHFR* 677C>T polymorphism may have a little effects on the incidence of IS [19].

Additionally, inflammation is considered to increase the risk of cardiovascular and celebrovascular diseases [20, 21]. As one of inflammatory markers, CRP is an important component of natural immune system in human body [22, 23]. *CRP* will surge sharply after damage or inflammation of the tissue to effectively exert its effects on immune protection [24].

Studies have discovered that *CRP* plays an important role in the occurrence and development of IS [25, 26], and serum CRP level can be regarded as a predictor for first-onset and recurrence of IS [27-29]. The study of Ballantyne et al. has showed that higher serum hs-CRP concentration makes people more vulnerable to IS and it is related to the functional and neurological disabilities [30]. The present study also showed that hs-CRP level in IS patients was higher than that of controls.

As for *CRP* polymorphisms, the study by Morita et al. showed there was no apparent association of rs1341665, rs1130864 and rs1205 with IS risk [31]. The research of Kuhlenbaeumer et al. also discovered that rs3093075, rs1205, rs1130864, rs1899947 and their haplotypes in *CRP* gene have no relationship with IS risk [32]. As shown in this study, the frequencies of genotypes and alleles of rs1130864, rs3093059 and rs1205 in *CRP* gene showed no statistically significant difference between two groups. Haplotype C-C-C was associated with the increased risk of IS. Based on the above results, we concluded that hs-CRP level was a risk factor for IS, while rs1130864, rs3093059 and rs1205 in *CRP* might work together in the occurrence of IS.

The occurrence and development of IS is a complicated process involving multiple genes working coordinately [33]. This study shows that there is no apparent association between *CRP* polymorphisms and IS susceptibility. The occurrence of IS may be related to multiple genes or synergistic effect of more polymorphisms in *CRP* gene. Further study with large sample size is needed to verify this result, in the meanwhile, the interactions of gene-gene, multiple polymorphisms in same or different genes and gene-environment should be considered.

Disclosure of conflict of interest

None.

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