Int J Clin Exp Med 2014;7(9):2858-2864
www.ijcem.com /ISSN:1940-5901/IJCEM0001748

Original Article

Interleukin-6 promotor polymorphisms and coronary vasospastic angina in Han Chinese

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Received August 6, 2014; Accepted August 28, 2014; Epub September 15, 2014; Published September 30, 2014

Abstract: There is an accumulating body of evidence indicating association between inflammation and the pathogenesis of coronary vasospastic angina (CVA). Interleukin-6 (IL-6) is a pleiotropic cytokine, functions as a mediator of inflammatory response and has both pro-inflammatory and anti-inflammatory properties. The aim of the present study is to investigate the association of -634C/G polymorphism of IL-6 gene with CVA in Han Chinese. A total of 27 CVA patients and 232 healthy controls were eligible for this study. The PCR-based restriction fragment length polymorphism (PCR-RFLP) technique was used to assess the genotypes frequencies. The distribution of the IL-6 -634C/G genotypes (CC, CG, and GG) was 59.48%, 37.07%, and 3.45% in the controls, and 37.04%, 48.15%, and 14.81% in CVA group, respectively (P = 0.0080). The frequency of the G allele in the CVA group was significantly higher than that in the control group (38.89% vs 21.98%, P = 0.0057). Compared with the wild type CC, the G allele carriers (CG + GG genotypes) had increased risk of CVA in both unadjusted and adjusted analyses. These findings suggest that IL-6 -634C/G polymorphism is associated with CVA and the G allele is an independent risk for CVA in Han Chinese.

Keywords: Interleukin-6, genetic polymorphism, coronary vasospastic angina, Chinese

Introduction

Coronary vasospastic angina (CVA) plays an important role in the pathogenesis of a variety of ischemic heart disease, including not only variant angina but also rest angina, effort angina, acute myocardial infarction, and other related conditions [1-3]. CVA is also an important cause of out-of-hospital cardiac arrest [4]. Although the precise mechanisms responsible for the occurrence of CVA are not known, several lines of evidence support a strong association between inflammation and the pathogenesis of CVA.

Histological studies involving coronary plaques in patients with coronary spasm have demonstrated evidence of intimal injury, such as neointimal hyperplasia with infiltration of inflammatory cells [5]. In addition, the peripheral monocyte count was reported as an independent marker for predicting CVA in the patients with resting chest pain [6]. Furthermore, some studies have shown that concentrations of inflammatory mediators or markers, such as interleukin (IL)-6 and high-sensitivity C-reactive protein (hs-CRP), were increased in patients with CVA and were correlated with CVA severity [7-10].

IL-6 is a pleiotropic cytokine of 23.7 kDa, functions as a mediator of inflammatory response and has both pro-inflammatory and anti-inflammatory properties [11-16]. Circulating levels of IL-6 differ greatly between individuals due to both genetic and environmental influences. Three single nucleotide polymorphisms (SNPs) in the IL-6 promoter region (-597G/A (rs1800797); -634C/G (rs1800796) and -174G/C (rs1800795)) have been reported to influence IL- 6 transcription, and -174G/C was in tight linkage disequilibrium with -597G/A. The -174C allele is extremely rare and the -634C allele is common in eastern Asian populations, whereas in Caucasians the -174C allele is relatively frequent and the -634C allele is less frequent. Based on these findings, we carried out a case-control study of the IL-6 -634C/G polymorphism to evaluate its putative association with CVA in Han Chinese patients with angina.

Table 1. Clinical characteristics of CVA and control subjects

Characteristics	CVA (n = 27)	Controls (n = 232)	*P* value
Age (years)	46.79 ± 11.82	51.37 ± 13.24	0.0868
Gender (% male)	51.85	61.64	0.3246
SBP (mmHg)	114.38 ± 21.36	123.46 ± 23.75	0.0587
DBP (mmHg)	75.43 ± 9.64	79.51 ± 10.39	0.0529
BMI (Kg/m^2)	23.49 ± 1.65	23.13 ± 1.58	0.2657
LVEF (%)	60.12 ± 6.57	62.38 ± 6.84	0.1041
TC (mmol/L)	5.05 ± 0.72	4.86 ± 0.60	0.1288
LDL-C (mmol/L)	2.63 ± 0.88	2.54 ± 0.52	0.4357
HDL-C (mmol/L)	1.40 ± 0.28	1.46 ± 0.32	0.3516
TG (mmol/L)	1.57 ± 0.55	1.66 ± 0.68	0.5082
IL-6 (pg/mL)	3.18 ± 0.88	2.66 ± 0.73	0.0007

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; LVEF, left ventricular ejection fraction; TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; TG, triglycerides; IL-6, interleukin-6.

Subjects and methods

Study subject

A total of 27 CVA patients were eligible for this study. Criteria [2, 3] for enrollment into the CVA group included: 1) an angina-like attacks at rest, during effort, or during rest and effort, and the following ischemic change findings are obtained by electrocardiogram (ECG), dynamic electrocardiography (DCG) recording during spontaneous attacks: a transient ST elevation of 0.1 mV or more, an ST depression of 0.1 mV or more, or new appearance of negative U waves, recorded in at least two contiguous leads on the 12-lead ECG; 2) an attack relieved by sublingual administration of nitroglycerin; 3) no significant obstructive coronary artery disease (<50% luminal narrowing of major coronary arteries) after intra-coronary nitroglycerin. The 232 healthy subjects in the corresponding period were recruited at their annual health examination, and did not have any chest symptoms or ECG abnormalities suggesting coronary artery disease (CAD), or a medical history of CAD. All study participants were enrolled at the Affiliated Hospital of Nantong University. Details of medical history, family history, and clinical symptoms were obtained from all participants using a standardized questionnaire, together with information of drug intake and cigarette smoking. Blood pressure, weight, height, and waistline were measured by trained physicians or nurses according to standardized protocols. Patients with acute coronary syndrome, hypertrophic cardiomyopathy, significant valvular disease, left ventricular dysfunction (ejection fraction <50%), and neoplastic, renal, liver, or thyroid diseases were excluded. All study subjects were unrelated Han nationality resident. The study was approved by the Medical Ethics Committee of Nantong University, and written informed consent was obtained from all participants.

Biochemical analysis

Venous blood samples were obtained after at least a 10-hour overnight fast and then centrifuged at 2500 rpm for 30 minutes at 4°C and immediately stored at -80°C until analysis. Measurement of total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), triglycerides (TG), hs-CRP, and IL-6 was performed as described previously [13, 17].

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes by the salting-out method with minimal modifications. Determination of IL-6 -634C/G genotypes was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) as described previously [11, 13].

Statistical analysis

All continuous variables are expressed as the mean and standard deviation (SD). Student's t-test was used to compare continuous variables from two groups. Genotypes and allele frequencies were obtained by direct count. Differences in the distribution of alleles and genotypes between the groups, and deviations from the Hardy-Weinberg equilibrium were assessed by χ^2 test. All significant tests were two-tailed and were considered statistically significant at $p < 0.05$. SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

The clinical characteristics of all participants enrolled in the study are given in **Table 1**. No significant differences were seen between the

Table 2. Distribution of the IL-6 -634C/G genotypes and alleles in CVA and control subjects

Groups	n	Genotypes frequencies (n, %)			Alleles frequencies (n, %)	
		CC	CG	GG	C	G
controls	232	138 (59.48)	86 (37.07)	8 (3.45)	362 (78.02)	102 (21.98)
CVA	27	10 (37.04)	13 (48.15)	4 (14.81)	33 (61.11)	21 (38.89)
P value			0.0080		0.0057	

Table 3. Relative risk of CVA according to IL-6 -634C/G genotypes

Genotypes	OR (95% CI)	*P* value	OR[a] (95% CI)	*P* value
CC	1.00		1.00	
CG	2.086 (0.8763-4.9657)	0.0914	2.0091 (0.8443-4.7808)	0.1096
GG	6.9 (1.769-26.9134)	0.0123	4.9091 (1.3215-18.2367)	0.0289
CG + GG	2.4957 (1.0949-5.6888)	0.0256	2.2838 (1.0026-5.2026)	0.0447

[a]Adjusted for age, gender, blood pressure, BMI, serum levels of lipids and IL-6.

two groups with regard to age, gender, body mass index (BMI), left ventricular ejection fraction (LVEF), and serum lipids levels. However, compared to the controls, CVA patients had higher serum IL-6 levels (P = 0.0007). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) despite a lower trend in the CVA patients did not differ substantially in the two groups.

The distributions of IL-6-634 C/G genotypes and allele frequencies for two groups are depicted in **Table 2**. The genotypes frequencies did not deviate significantly from those predicted by Hardy-Weinberg equilibrium in both the control group (χ^2 = 1.5111, P = 0.2190) and the CVA subjects (χ^2 = 0.0046, P = 0.9462). The distribution of the IL-6 -634C/G genotypes (CC, CG, and GG) was 59.48%, 37.07%, and 3.45% in the controls, and 37.04%, 48.15%, and 14.81% in CVA group, respectively (P = 0.0080). The frequency of the G allele in the CVA group was significantly higher than that in the control group (38.89% vs 21.98%, P = 0.0057). Compared to the wild type CC, the G allele carriers (CG + GG genotypes) had a 2.4957-fold increased risk of CVA (odds ratio [OR] = 2.4957, 95% confidence interval [CI] = 1.0949-5.6888, P = 0.0256). After being adjusted for age, gender, blood pressure, BMI, serum levels of lipids and IL-6, the association persisted (adjusted OR = 2.2838, 95% CI = 1.0026-5.2026, P = 0.0447). Seen **Table 3**.

Table 4 summarizes the effects of the different genotypes on clinical parameters. The carriers of the G allele (CG + GG) were pooled into one group, because the numbers of individuals with the GG genotype were small. Different genotypes of IL-6 had no effects on age, gender, SBP, DBP, BMI, LVEF, and serum lipids levels either in the CVA or control groups. However, subjects with the CG + GG genotype had higher IL-6 levels than did patients with the CC genotype both in the CVA group (P = 0.0303) and the controls (P = 0.0240).

Discussion

The major finding of the present study is the significant association of the IL-6 -634C/G polymorphism with risk of developing CVA. Compared with the wild type CC, the G allele carriers (CG + GG genotypes) had a 2.4957-fold increased risk of CVA. The G allele carriers also had higher serum IL-6 levels compared with the CC homozygotes in both CVA and control group. These finding support the hypothesis that low-grade inflammation plays a role in the underlying mechanisms of CVA.

In the past few years, much attention has been devoted to assess the role of IL-6 in CVA [18]. IL-6 is one of the most important mediators of the acute-phase response and a primary determinant of hepatic production of C-reactive protein [19]. IL-6 not only contributed to the inflammatory response by activating endothelial cells, stimulating the synthesis of fibrinogen [20, 21], but was shown to be associated with markers of endothelial dysfunction such as chemokine and adhesion molecule release as well [22, 23]. Endothelial dysfunction has been considered to play an important role in CVA [3, 18, 24]. Furthermore, elevated circulating levels of IL-6 and hs-CRP have been significantly associ-

Table 4. Clinical parameters according to different genotypes in CVA and control subjects

Characteristics	CVA			Controls		
	CC (n = 10)	CG + GG (n = 17)	*P* value	CC (n = 138)	CG + GG (n = 94)	*P* value
Age (years)	46.89 ± 11.94	46.73 ± 11.85	0.9733	51.68 ± 14.52	50.91 ± 13.66	0.6851
Gender (% male)	50.00	52.94	0.8826	62.32	60.64	0.7961
SBP (mmHg)	113.17 ± 21.02	120.97 ± 23.13	0.8788	122.25 ± 23.61	125.24 ± 23.80	0.3462
DBP (mmHg)	74.82 ± 9.25	75.79 ± 10.01	0.6609	78.91 ± 10.23	80.39 ± 10.52	0.2860
BMI (Kg/m^2)	23.48 ± 1.61	23.50 ± 1.67	0.4945	23.16 ± 1.67	23.08 ± 1.42	0.7042
LVEF (%)	59.83 ± 6.49	60.29 ± 6.64	0.5008	62.34 ± 6.81	62.44 ± 6.89	0.9131
TC (mmol/L)	4.94 ± 0.66	5.11 ± 0.74	0.0979	4.82 ± 0.53	4.93 ± 0.62	0.1490
LDL-C (mmol/L)	2.55 ± 0.81	2.68 ± 0.90	0.0722	2.56 ± 0.51	2.51 ± 0.46	0.4466
HDL-C (mmol/L)	1.43 ± 0.26	1.38 ± 0.25	0.3028	1.43 ± 0.29	1.50 ± 0.33	0.0893
TG (mmol/L)	1.63 ± 0.58	1.53 ± 0.51	0.3219	1.63 ± 0.58	1.70 ± 0.69	0.4045
IL-6 (pg/ml)	2.68 ± 0.73	3.47 ± 0.93	0.0303	2.57 ± 0.64	2.78 ± 0.76	0.0240

ated with the prevalence and severity of CVA [7-10]. Published studies have shown that IL-6, as a principal upstream mediator of the acute-phase proteins, regulates CRP gene expression [19]. The association of IL-6 polymorphism with higher serum hs-CRP concentration maybe due to an increase in IL-6 gene transcription and the consequent effects of increasing levels of IL-6 on CRP gene expression [13]. Our previous report [13] along with the studies conducted in Japanese [25] and Koreans [26] indicated that IL-6-634G allele was associated with higher circulating levels of IL-6 and CRP in eastern Asians.

It has been suggested that CVA is more prevalent in eastern Asian populations than in white populations, although there were differences in both the route and dose level of administration of CVA inducers used [27]. Eastern Asian patients with recent myocardial infarction have greater coronary vasoreactivity than their Caucasian counterparts, and spasm is more important in the pathogenesis of myocardial infarction in eastern Asian patients [28]. Provocative vasomotor studies in Japanese population with acute coronary syndrome without a culprit lesion, 79% had a positive result [29], whereas 16% of French [30] and 49% of similar German [31] patients developed CVA after intracoronary acetylcholine. Racial difference suggested an important role of genetic factors in the pathogenesis of CVA. Several investigations have been trying to unravel some of these genetic backgrounds with the use of association studies. In some case–control studies, genetic variations of endothelial nitric oxide synthase (eNOS) Glu298Asp [32], -786T/C [33, 34], 4b/a [35], phospholipase C-δ1 (PLC-δ1) R257H [36], NADH/NADPH oxidase p22phox gene 242C→T [23], paraoxonase Gln192Arg [37], manganese superoxide dismutase (MnSOD) Ala16Val [38], stromelysin-1 gene -1171/5A→6A [23], and rho-associated Kinase (ROCK) 2 GTCTG haplotype [39], are identified as risk factors for CVA. However, most of these genetic variations could not explain the distribution of CVA with respect to ethnic origin. But IL-6 genotypes distribution is associated with ethnicity. Among the three SNPs in the IL-6 gene promoter region, -174G/C is in tight linkage disequilibrium with -597G/A [40], and the -174C allele is extremely rare and the -634C allele is common in eastern Asian populations, whereas in Caucasians the -174C allele is relatively frequent and the -634C allele is less frequent [13, 41]. Thus, our data will provide an important clue to clarify the ethnicity association of CVA.

Our study has some potential limitations. Firstly, the cross-sectional study with no prospective data limits our ability to extract conclusions about the temporal relationships of inflammation and AF. The relatively limited cohort size restricts the generalizability of our results. Secondly, although coronary angiography is liberally used in China, invasive provocation tests for CVA is still regarded as difficult and fraught with complications [18, 27]. However, in the present study, our diagnostic criteria for CVA was according to the Japanese Circulation Society (JCS) guidelines for diagnosis and treatment of patients with vasospastic

angina (coronary spastic angina) [3]. Finally, 232 healthy subjects with no signs of angina pectoris were enrolled in the present study, but we did not demonstrate that they did not have atherosclerotic coronary artery disease or CVA by examinations such as exercise test or CAG. Thus, we could not exclude the presence of previous asymptomatic CVA in the control group. A prospective study with larger sample size comparing the CVA patients and control subjects rigorously diagnosed is necessary to obtain more convincing evidence.

In conclusion, our data indicate that IL-6 -634C/G polymorphism is associated with CVA and the G allele is an independent risk for CVA in Han Chinese. Our results provide an important clue to clarify the role of inflammation in CVA and the distribution of CVA with respect to ethnic origin.

Acknowledgements

This work was supported by the Clinical Medicine Special Foundation of Nantong City (HS2013069), and "Summit of the Six Top Talents" Program of Jiangsu Province (2009046), P. R. China.

Disclosure of conflict of interest

None.

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