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IL-6 gene promoter polymorphisms and risk of coronary artery disease in a Chinese population

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ABSTRACT. We investigated the relationships between single nucleotide polymorphisms (SNPs) of the interleukin (IL)-6 gene 174 G>C (rs1800795), 572 G>C (rs1800796), and 597 G/A (rs1800797) and coronary artery disease (CAD) risk in a Chinese population. This case-control study recruited 296 CAD patients and 327 controls between January 2009 and May 2012. Genotyping of *IL-6* 174 G>C (rs1800795), 572 G>C (rs1800796), and 597 G/A (rs1800797) was performed on a 384-well plate format using the Sequenom MassARRAY platform. CAD patients were more likely to be older and male, with a higher body mass index, diabetes, and hypertension, and presented higher triglycerides, and lower total cholesterol, low-density lipoprotein-cholesterol, and high-density lipoprotein-cholesterol. We found that the *IL-6* 174CC genotype was associated with a significantly increased risk of CAD compared to the wild-type GG genotype in a codominant model [odds ratio (95% confidence interval) = 1.94 (1.13-

3.37)], whereas *IL-6* 174 G>C polymorphisms presented an increased risk of CAD in dominant and recessive models. However, we did not find that the *IL-6* 572 CC and 597 AA genotypes were correlated with an increased risk of CAD. *IL-6* 174 G>C rs1800795 was associated with CAD risk in a Chinese population. Further large-scale studies are required to determine whether *IL-6* SNPs interact with environmental factors in the development of CAD.

Key words: Coronary artery disease; Chinese population; Interleukin-6; Case-control study

INTRODUCTION

Coronary artery disease (CAD) is a common and fatal chronic disease. More than half of cardiovascular events in men and women occur in patients under the age of 75 years (Lloyd-Jones et al., 2010). CAD has a complex etiology determined by factors such as inflammation, gender, age, smoking, hypertension, diabetes, and genetic susceptibility (Ross, 1999; Paoletti et al., 2004). The underlying pathological process of CAD is atherosclerosis, which is characterized by chronic inflammation due primarily to the deposit of oxidized lipids on the inner layer of the arterial wall. Studies have indicated that inflammation-related genes may be associated with CAD risk (Xie et al., 2012; Li et al., 2012; Kroeger et al., 2012; Kim et al., 2012; LaFramboise et al., 2012).

Inflammation plays a key role in the pathogenesis of atherosclerosis (Mehta et al., 1998; Hansson, 2005). As a pleiotropic inflammatory cytokine, interleukin (IL)-6 plays an important role in the acute-phase response and inflammatory cascade by upregulating acute-phase proteins such as C-reactive protein (Nabata et al., 1990; Yudkin et al., 1999). The *IL-6* gene is located on chromosome 7p21. Three single nucleotide polymorphisms (SNPs), 174 G>C (rs1800795), 572 G>C (rs1800796), and 597 G/A (rs1800797), have been widely investigated because of their association with the risk of various diseases (Rasmussen et al., 2013). However, the results are inconsistent. In addition, few studies have been conducted in China regarding the association of these polymorphisms with CAD. Therefore, we investigated the relationships between SNPs of *IL-6* 174 G>C (rs1800795), 572 G>C (rs1800796), and 597 G/A (rs1800797) and CAD risk in a Chinese population.

MATERIAL AND METHODS

Study population

This case-control study recruited 328 patients first diagnosed with CAD at the First Hospital of Jilin University between January 2009 and May 2012. Inclusion criteria were angiographic evidence of ≥70% stenosis of one major coronary artery or ≥50% stenosis of the left main coronary artery. Exclusion criteria included current heparin treatment, autoimmune disease, congenital heart disease, severe kidney or liver disease, or malignancy. Three hundred sixty-two control subjects were initially recruited for the study, and those with known CAD or any other heart disease were excluded from participation. A total of 296 patients were involved in our study, with a participation rate of 90.2%.

Demographic and clinical characteristics were collected from medical records. All patients were asked to provide 5 mL venous blood, collected in ethylenediaminetetraacetic acid-coated tubes and stored at -20°C until use. Body mass index (BMI), hypertension, and diabetes data were collected from medical records. Total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels were determined based on serum tests.

Genotyping

Genomic DNA was extracted using the method of buffy coat fractions with the TIANamp blood DNA kit (Tiangen Biotech; Beijing, China). Genotyping of *IL-6* 174 G>C (rs1800795), 572 G>C (rs1800796), and 597 G/A (rs1800797) was performed on a 384-well plate format on the Sequenom MassARRAY platform (Sequenom; San Diego, CA, USA), which involved polymerase chain reaction (PCR) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Figure 1). Single-base extension (SBE) and PCR primers were designed using the Sequenom Assay Design 3.1 software (Sequenom). Each PCR reaction was carried out in a volume of 20 μL containing 50 ng genomic DNA, 200 μM dNTPs, 2.5 U *Taq* DNA polymerase, and 200 μM primers. The cycling program for the PCR reaction was preliminary denaturation at 94°C for 2 min followed by 35 cycles of 94°C for 30 s, annealing at 64°C for 30 s, and 72°C for 10 min. The PCR product was evaluated by 1.0% agarose gel electrophoresis. A repeat analysis of a randomly chosen subgroup of 10% of the cases and controls was conducted for quality control; the reproducibility was 100%.

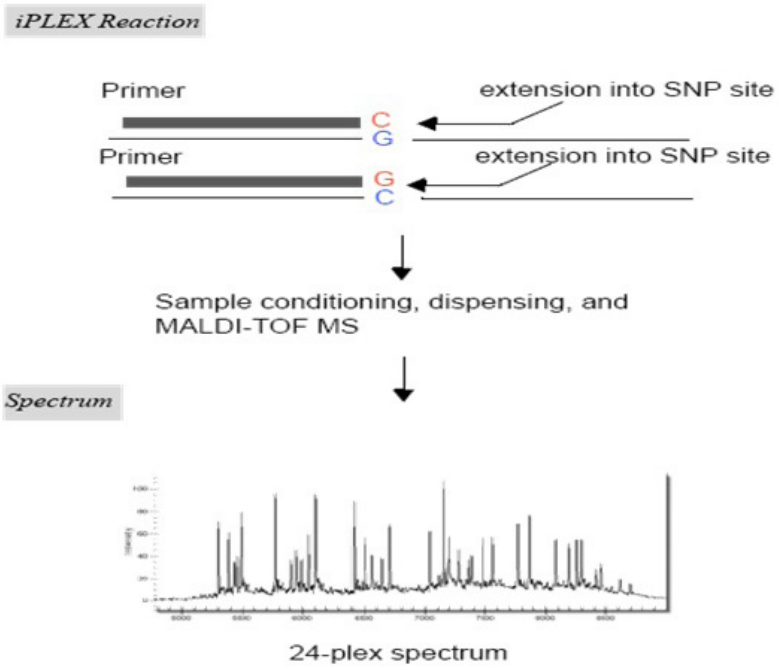

Figure 1. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry technique.

Statistical analysis

Continuous variables are shown as the mean ± SD and were analyzed using the Student *t*-test. Categorical variables are expressed as the frequency and percentage and were analyzed using the chi-square test. Hardy-Weinberg equilibrium and genotype distributions between groups were analyzed using the chi-square test. Odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) were determined to evaluate the effect of *IL-6* 174 G>C (rs1800795), 572 G>C (rs1800796), and 597 G/A (rs1800797) on the risk of CAD. Multivariable logistic regression analysis was conducted to calculate the OR (95%CI) after adjusting for potential confounding factors. All statistical analyses were conducted using the SPSS 11.0 software (SPSS, Inc.; Chicago, IL, USA), and $P < 0.05$ was considered to be statistically significant.

RESULTS

This study included 296 CAD cases (205 males and 91 females, mean age of 61.2 ± 8.5 years) and 327 healthy controls (182 males and 145 females, mean age of 56.4 ± 11.6 years) (Table 1). Compared with controls, CAD patients were more likely to be of older age and male. Patients who had higher BMI and suffered from diabetes and hypertension were more likely to have CAD. Patients with higher TG and lower TC, LDL-C, and HDL-C had a higher risk of CAD ($P < 0.05$ for all comparisons).

Table 1. Clinical characteristics of coronary artery disease patients and controls.

Variables	Cases	%	Controls	%	χ^2 or *t*	P value
	N = 296		N = 327			
Age (years)	61.2 ± 8.5		56.4 ± 11.6		5.84	<0.001
Gender						
Male	205	69.3	182	55.7		
Female	91	30.7	145	44.3	12.21	<0.001
BMI (kg/m^2)						
<24	120	40.6	172	52.6		
≥24	176	59.4	155	47.4	9.07	0.003
Diabetes						
No	104	35.2	28	8.6		
Yes	192	64.8	299	91.4	65.70	<0.001
Hypertension						
No	88	29.7	61	18.6		
Yes	208	70.3	266	81.4	10.47	0.001
TC (mM)	4.3 ± 0.9		4.5 ± 1.2		2.33	0.01
TG (mM)	2.1 ± 1.0		1.8 ± 1.1		3.55	<0.001
LDL-C (mM)	2.4 ± 0.9		3.0 ± 0.9		8.31	<0.001
HDL-C (mM)	1.3 ± 0.5		1.6 ± 0.5		7.48	<0.001

BMI = body mass index; TC = total cholesterol; TG = triglycerides; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.

The genotype distributions of the three SNPs are presented in Table 2. In the control subjects, the distributions of *IL-6* 174 G>C rs1800795, 572 G>C rs1800796, and 597 G/A rs1800797 were in line with Hardy-Weinberg equilibrium. The 572 G>C rs1800796 and 597 G/A rs1800797 genotype frequencies were not significantly different between the CAD and control groups, whereas the *IL-6* 174CC genotype was significantly different between the two groups ($P < 0.05$).

Table 2. Multivariate logistic regression analysis of the effects of interleukin-6 on risk of coronary artery disease.

Genotype	Minor allele		P value	OR (95%CI)[a]		
	Cases	Controls		Codominant	Dominant	Recessive
rs1800795						
GG	191	236		-	-	-
GC	61	63		1.20 (0.79-1.82)	1.43 (1.01-2.03)	1.86 (1.10-3.20)
CC	44	28	0.03	1.94 (1.13-3.37)		
rs1800796						
GG	190	215		-	-	-
GC	69	73		1.07 (0.72-1.60)	1.07 (0.76-1.51)	1.05 (0.63-1.75)
CC	37	39	0.82	1.08 (0.64-1.81)		
rs1800797						
GG	186	214		-	-	-
GA	78	82		1.09 (0.75-1.61)	1.12 (0.80-1.58)	1.16 (0.66-2.02)
AA	32	31	0.92	1.19 (0.67-2.10)		

[a]Adjusted for sex, gender, body mass index, diabetes, hypertension, and levels of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides.

Multivariate logistic regression analysis was conducted to analyze the effects of *IL-6* 174 G>C rs1800795, 572 G>C rs1800796, and 597 G/A rs1800797 on CAD risk, adjusting for gender, age, BMI, diabetes, hypertension, and levels of TC, HDL-C, LDL-C, and TG. We found that the *IL-6* 174 CC genotype was associated with a significantly increased risk of CAD compared to the wild-type GG genotype in a codominant model [OR (95%CI) = 1.94 (1.13-3.37)], and *IL-6* 174 G>C polymorphisms presented an increased risk of CAD in dominant and recessive models [OR (95%CI): 1.43 (1.01-2.03) and 1.86 (1.10-3.20), respectively]. In addition, we found that the *IL-6* 572 CC and 597 AA genotypes were correlated with a slightly increased risk of CAD, but no statistical significance was found between them ($P > 0.05$).

DISCUSSION

In our study, we found that the *IL-6* 174 G>C rs1800795 polymorphism was associated with CAD risk in a Chinese population, but there were no associations between polymorphisms in 572 G>C rs1800796 and 597 G/A rs1800797 and risk of CAD. We found that the effects of the *IL-6* 174 CC genotype and C allele increased the risk of CAD in the Chinese population examined in this study.

The *IL-6* gene is located on chromosome 7p21; IL-6 is a multifunctional cytokine produced by immune and many non-immune cells and functions both as an inflammatory mediator and an endocrine and metabolic function regulator. A previous study indicated that IL-6 is one of the most important mediators of the *in vivo* inflammatory reactions associated with CAD, and is likely to be a key mediator in the inflammatory response to CAD (Su et al., 2013; Anderson et al., 2013; Phulukdaree et al., 2013). We found that *IL-6* 174 G>C was associated with an increased risk of CAD, suggesting that *IL-6* is a key regulator of inflammatory mechanisms that play an important role in the pathophysiology and development of CAD. A previous study conducted in South Africa showed that the *IL-6* 174 G allele influences mRNA levels and protein expression in CAD, and reduced the risk of CAD, with an OR (95%CI) of 0.47 (0.23-0.95) (Phulukdaree et al., 2013). A meta-analysis focusing on the *IL-6* 174G/C and -572G/C variants indicated that the association between the *IL-6* gene and CAD risk was mild and moderate for these polymorphisms (Yang et al., 2013). These results agree with those of our study. However, some studies have reported inconsistent results. A study conducted in

Tunisia with 418 CAD patients and 406 controls indicated that the *IL-6* 174G/C variant is not associated with an increased risk of CAD (Ghazouani et al., 2011). Another Turkish cohort study found that the *IL-6* 174G/C polymorphism does not contribute to the risk of cardiovascular disease (Sekuri et al., 2007).

The strengths of this study include the fact that the allele frequencies for all *IL-6* polymorphisms examined were similar to those previously reported for the Chinese population (Chen et al., 2013; Shi et al., 2013). However, there were several limitations to our study. First, cases and controls were not age- or gender-matched, but we used further adjustments to minimize potential biases. Second, some risk factors of CAD were not included in the analysis. Therefore, large population-based studies including subjects of different ethnicities are warranted to further investigate the impact of *IL-6* polymorphisms on CAD susceptibility.

In conclusion, we found that *IL-6* 174 G>C rs1800795 polymorphisms are associated with CAD risk in a Chinese population. The 572 G>C rs1800796 and 597 G/A rs1800797 polymorphisms were not associated with CAD risk in this population. Further large-scale studies are required to determine whether these IL-6 SNPs interact with environmental factors in the development of CAD.

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