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Role of interleukin-6 gene polymorphisms in the risk of coronary artery disease

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ABSTRACT. We conducted a case-control study to investigate the association between *IL-6* -174 G>C and -572 C>G polymorphisms and the risk of coronary artery disease (CAD). We genotyped *IL-6* -174 G>C and -572 C>G in 402 patients with CAD and 402 control individuals. *IL-6* -174 G>C (rs1800795) and -572 C>G (rs1800796) alleles were detected by polymerase chain reaction-restriction fragment length polymorphism. Patients with CAD were more likely to have a smoking habit, diabetes, and hypertension, a high level of triglycerides, and low levels of total cholesterol and high- and low-density lipoprotein cholesterol. Multivariate regression analyses showed that subjects carrying the *IL-6* -174CC genotype had a small but significant increased risk of CAD (P = 0.004). Those carrying the *IL-6* -174 G>C polymorphic variant had a slightly increased risk of CAD in both dominant and recessive models. However, we did not find significant association between the *IL-6* -572 C>G polymorphism and risk of CAD. Moreover, a significant interaction was found between the *IL-6* -174 G>C polymorphism, gender, and smoking habit. Our study, therefore, demonstrated that the *IL-6* -174 G>C polymorphism is correlated with

CAD risk, and that this polymorphism shows interactions with both gender and smoking.

Key words: Interleukin-6; Polymorphism; Coronary artery disease

INTRODUCTION

Coronary artery disease (CAD) is a common and fatal chronic disease with high mortality. It is estimated that there have been 17.5 million deaths every year worldwide, with most of the cardiovascular events having occurred below the age of 75 years (Pignone et al., 2010). Previous studies have shown that CAD is caused by various factors, such as inflammation, gender, age, smoking, soy food intake, hypertension, and diabetes, as well as hereditary factors (Sayols-Baixeras et al., 2014; Yiannakouris et al., 2014; Yu et al., 2014). The underlying pathological mechanism of CAD is atheroma plaque instability, which is characterized by chronic inflammation caused by oxidized lipids adherent on the inner layer of the arterial wall. Recent studies have shown that inflammation-related genes might be correlated with CAD risk (Kroeger et al., 2012; Li et al., 2012; Xie et al., 2012).

Interleukin-6 (IL-6) is a proinflammatory and immunoregulatory cytokine found in diverse tissues, including fibroblasts, monocytes, adipocytes, and endothelial cells. IL-6 has a role in the genesis and maintenance of the inflammatory response (Balding et al., 2004). The polymorphisms of the *IL-6* gene are associated with different levels of secreted protein according to the genotype. Two functional variants in the *IL-6* gene, -174 G>C (rs1800795) and -572 C>G (rs1800796), have been widely investigated with relation to their association with risk of various disease (Mandić et al., 2013; Nie et al., 2014; Sung et al., 2014; Yang et al., 2014). These two gene polymorphisms may influence CAD susceptibility by altering gene regulation and protein expression (Morgan et al., 2006). Several studies show that *IL-6* gene polymorphisms are associated with risk for CAD, but different studies have reported conflicting results (Bhanushali and Das, 2013; Cui et al., 2013; Satti et al., 2013). Therefore, we conducted a case-control study to investigate the association between *IL-6* -174 G>C and -572 C>G polymorphisms and risk of CAD.

MATERIAL AND METHODS

Study population

A total of 433 Chinese patients who were initially diagnosed with CAD were selected from the First Affiliated Hospital of He'nan University, Xinxiang, China. All patients were diagnosed by angiographic evidence of ≥70% stenosis of one major coronary artery, or ≥50% stenosis of the left main coronary artery. Patients who had autoimmune disease, congenital heart disease, severe kidney or liver disease, or malignancy were excluded from our study. In total, 402 patients were selected for our study, with a participation rate of 92.8%. A total of 402 age- and gender-matched controls were collected from subjects who had taken a health examination as part of our study. Control subjects who suffered from CAD or any other heart disease were excluded from the study. All patients and control subjects signed a written informed consent form. Our study was approved by the Ethics Committee of the First Affiliated Hospital of He'nan University.

A structured questionnaire was used to collect general information from patients and controls, including age, gender, smoking and drinking habits, hypertension, diabetes, and obesity. Total cholesterol (TC), triglycerides (TG), and low- and high-density lipoprotein cholesterol (LDL-C and HDL-C, respectively) levels were determined by serum analysis.

Genotyping assays

All study subjects were asked to provide 5 mL peripheral venous blood. Genomic DNA was extracted from peripheral venous blood samples using the TIANamp Blood DNA kit according to manufacturer instructions (Tiangen Biotech, Beijing, China). Genotyping analyses of *IL-6* -174 G>C and -572 C>G were performed by polymerase chain reaction-restriction fragment length polymorphism. The primers used for *IL-6* -174 G>C and -572 C>G were designed using the Sequenom Assay Design 3.1 software (Sequenom®, San Diego, CA, USA) according to manufacturer instructions. The forward and reverse primers for *IL-6* -174 G>C were 5'-TGA CTT CAG CTT TAC TCT TTG T-3' and 5'-CTG ATT GGA AAC CTT ATT AAG-3', respectively. The forward and reverse primers for *IL-6* -572 C>G were 5'-AGA TTC CAA GGG TCA CTT G-3' and 5'-AGA AGC AGA ACC ACT CTT C-3', respectively. The cycling program involved preliminary denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 10 min. For quality control, 5% of subjects were randomly selected, and the results of repeated samples showed 100% concordance.

Statistical analysis

Continuous variables are reported as means ± SD, and categorical variables are reported as N of subjects (%). The continuous variables and categorical variables were analyzed using the χ^2 test. The Hardy-Weinberg equilibrium evaluation and between-group comparison of genotype distributions were carried out using the χ^2 test. The odds ratios (OR) and corresponding 95% confidence intervals (CIs) were calculated by conditional logistic regression analysis and utilized to assess the potential association between genotype frequencies and risk of CAD. The homozygous genotypes of the three single nucleotide polymorphisms were taken as the reference group. All P values were two sided, and $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using the SPSS version 11.0 software (SPSS Inc., Chicago, IL, USA) for Windows.

RESULTS

This study included 402 patients with CAD and 402 healthy controls, with 232 men and 170 women in each group, respectively. The mean ages of patients and controls were 65.4 ± 8.4 and 62.4 ± 8.5 years, respectively (Table 1). Patients with CAD were more likely to have a smoking habit, diabetes, and hypertension; and have a high TG level and low levels of TC, HDL-C, and LDL-C.

The genotype distributions of *IL-6* -174 G>C and -572 C>G in controls were in line with Hardy-Weinberg equilibrium, and P values were 0.24 and 0.12, respectively. Genotype frequencies for *IL-6* -174 G>C showed significant differences between patients and controls ($\chi^2 = 8.17$, $P = 0.017$), while frequencies of *IL-6* -572 C>G did not. Multivariate regression

analyses showed that subjects carrying the *IL-6* -174CC genotype had a small but significant increased risk of CAD, with an adjusted OR (95%CI) of 1.71 (1.13-2.60) (P = 0.004; Table 2). Similarly, we found that those carrying the *IL-6* -174 G>C polymorphism had a light increased risk of CAD in both dominant and recessive models, with adjusted ORs (95%CIs) of 1.43 (1.08-1.91) and 1.51 (1.02-2.24), respectively (Table 2). However, we did not find significant association between the *IL-6* -572 C>G polymorphism and risk of CAD.

Table 1. Clinical characteristics between patients with CAD and controls.

Variables	Patients (N)	%	Controls (N)	%	*t* or χ^2	P value
Age, years (mean ± SD)	65.4 ± 8.4		64.9 ± 8.2		0.85	0.2
Gender (%)						
Male	232	57.6	232	57.6	0.00	1.00
Female	170	42.4	170	42.4		
BMI (kg/m^2)		22.8 ± 2.9		22.6 ± 2.6	1.03	0.15
Smoking (%)						
Ever	154	38.2	131	32.6		
Never	248	61.8	271	67.4	4.82	0.03
Diabetes (%)						
Yes	127	31.7	74	18.5		
No	275	68.3	328	81.5	30.95	<0.001
Hypertension (%)						
Yes	111	27.6	78	19.4		
No	291	72.4	324	80.6	8.85	0.003
TC (mM)		4.2 ± 1.8		4.5 ± 1.6	2.57	0.005
TG (mM)		1.9 ± 1.3		1.7 ± 1.1	2.35	0.01
LDL-C (mM)		2.7 ± 1.2		2.9 ± 1.4	2.17	0.02
HDL-C (mM)		1.3 ± 0.6		1.4 ± 0.7	2.17	0.02

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

Table 2. Genotype frequencies of *IL-6* -174 G>C and -572 C>G among young patients with CAD and control subjects, and their odds ratios.

SNP		Patients	%	Controls	%	HWE	OR (95%CI)*		
		(N = 402)		(N = 402)			Codominant model	Dominant model	Recessive model
-174 G>C	GG	153	38.1	182	45.3		1.0 (Ref.)		
	GC	171	42.5	169	42.0		1.32 (0.96-1.82)		
	CC	78	19.4	51	12.7	0.24	1.71 (1.13-2.60)	1.43 (1.08-1.91)	1.51 (1.02-2.24)
-572 C>G	CC	176	43.8	192	47.8		1.0 (Ref.)		
	CG	187	46.5	181	45.0		1.10 (0.81-1.51)		
	GG	39	9.7	29	7.2	0.12	1.47 (0.86-2.56)	1.17 (0.88-1.56)	1.42 (0.84-2.42)

*Adjusted for age, gender, smoking, diabetes, hypertension, total cholesterol (TC), triglycerides (TG), low- and high-density lipoprotein cholesterol (LDL-C, HDL-C, respectively). SNP = single nucleotide polymorphism; HWE = Hardy-Weinberg equilibrium; OR = odds ratio; CI = confidence interval.

We further analyzed the interaction between the *IL-6* -174 G>C polymorphism and demographic factors on the risk of CAD. As there was a relatively small sample size of patients with the -174CC genotype, we combined patients with -174CC and GC genotypes as one group for analysis. When compared with the -174GG genotype, we found that male patients, and those who were "ever smokers", who carried the *IL-6* -174 GC + CC genotype had a higher risk of CAD, with ORs (95%CIs) of 1.49 (1.01-2.20) and 2.12 (1.28-3.52), respectively (Table 3). Moreover, a significant interaction was found between the *IL-6* -174 G>C

polymorphism and gender and smoking habits, and the P values for the interaction were 0.03 and 0.04, respectively. However, the *IL-6* -174 G>C polymorphism had no interaction with age, body mass index, diabetes, hypertension, TC, TG, LDL-C, or HDL-C.

Table 3. Association between the *IL-6* -174 G>C polymorphism and demographic factors on the risk of coronary artery disease (CAD).

Subgroup	*IL-6*-174 GG		*IL-6* -174 GC + CC		χ^2 value	P value	OR (95%CI)	P for interaction
	Patient	Control	Patient	Control				
Age (years)								
<65	75	93	126	107	3.48	0.06	1.46 (0.96-2.22)	
≥65	78	89	123	113	1.15	0.28	1.24 (0.82-1.88)	0.23
Gender								
Male	82	104	150	128	4.34	0.04	1.49 (1.01-2.20)	
Female	71	66	99	104	0.31	0.58	0.88 (0.56-1.40)	0.03
BMI								
<23	81	97	135	124	1.85	0.17	1.30 (0.87-1.95)	
≥23	72	85	114	96	2.55	0.11	1.40 (0.91-2.17)	0.35
Smoking								
Ever	61	54	93	77	9.56	0.002	2.12 (1.28-3.52)	
Never	92	128	156	143	0.1	0.76	1.06 (0.73-1.52)	0.04
Diabetes								
Yes	47	36	80	38	2.61	0.11	1.61 (0.86-3.00)	
No	106	146	169	182	2.19	0.14	1.28 (0.91-1.80)	0.11
Hypertension								
Yes	40	39	71	39	3.67	0.06	1.78 (0.94-3.34)	
No	113	143	178	181	1.78	0.18	1.24 (0.89-1.74)	0.14
TC (mM)								
<4.3	78	85	132	102	2.82	0.09	1.41 (0.93-2.15)	
≥4.3	75	97	117	118	1.52	0.22	1.28 (0.85-1.94)	0.19
TG (mM)								
<1.8	72	97	118	118	2.16	0.14	1.35 (0.89-2.04)	
≥1.8	81	85	131	102	2.15	0.14	1.35 (0.88-2.05)	0.47
LDL-C (mM)								
<2.8	71	86	116	105	1.94	0.16	1.34 (0.87-2.06)	
≥2.8	82	96	133	115	2.37	0.12	1.35 (0.90-2.03)	0.31
HDL-C (mM)								
<1.3	73	88	122	103	2.96	0.09	1.43 (0.93-2.19)	
≥1.3	80	94	127	117	1.5	0.22	1.28 (0.85-1.92)	0.52

BMI = body mass index; TC = total cholesterol; TG = triglycerides; LDL-C/HDL-C = low-/high-density lipoprotein cholesterol; OR = odds ratio; CI = confidence interval.

DISCUSSION

The identification of genes involved in the genetic predisposition or progression of disease has an important role in clinical practice and basic medical research. The use of genetic determinants for identifying high-risk populations and performance of targeted therapies based upon an individual's genetic make-up are well known clinical strategies. Many studies have investigated the genetic contribution of variants within the inflammatory cytokines to the development of CAD, but these have shown inconsistent results (Bhanushali and Das, 2013; Cui et al., 2013; Satti et al., 2013).

The *IL-6* gene is located on chromosome 7p21. IL-6 itself is a multifunctional cytokine produced by immune and many non-immune cells, with roles both as an inflammatory mediator and also as a regulator of endocrine and metabolic function. Several studies have found that IL-6 might be involved in the development of CAD (Ghazouani et al., 2011; Phulukdaree et al.,

2013; Satti et al., 2013; Tong et al., 2013), but the results have been inconsistent. Therefore, we conducted a case-control study to investigate the relationship between the two common polymorphisms of the *IL-6* gene and CAD, in order to gain a more reliable conclusion.

Our study found that the *IL-6* -174CC genotype was associated with an increased risk of CAD, and this increased risk was also found using both dominant and recessive models. Our findings are in line with results from previous studies (Phulukdaree et al., 2013; Satti et al., 2013). Satti et al. (2013) reported that the *IL-6* gene polymorphism was an independent risk factor for CAD. Another recent study conducted by Phulukdaree et al. (2013) reported that the *IL-6* -174G allele influenced the levels of IL-6 and the risk of CAD in South African Indians. However, two additional studies reported conflicting results (Ghazouani et al., 2011; Tong et al., 2013). Ghazouani et al. (2011) and Tong et al. (2013) reported that the *IL-6* -174 G>C variant was not correlated with an increased risk of CAD in Tunisians or in a Chinese population, respectively. However, a recent meta-analysis of 50 studies suggested that the *IL-6* -174 G>C polymorphism was positively associated with susceptibility to CAD (Yin et al., 2013), which is in line with the results of our study. The overall inconsistency of results between studies might be caused by the effects of different ethnicities, sample size, or case selection. Therefore, further ethnicity-specific studies are greatly needed to confirm our results.

In our subgroup analysis, we found that the *IL-6* -174 G>C polymorphism had interactions with gender and with smoking habits. IL-6 is a pleiotropic cytokine and a mediator of the inflammatory response, and functions as both a proinflammatory and anti-inflammatory molecule. Previous studies have suggested that the *IL-6* -174 G>C polymorphism has interactions with obesity and smoking (Panoulas et al., 2009; Franch-Chillida et al., 2010), which is partially inconsistent with the results from our study. Therefore, further large sample studies are greatly needed to confirm the association between *IL-6* -174 G>C polymorphism and CAD risk.

In conclusion, the results from our study suggest a correlation between the *IL-6* -174 G>C polymorphism and CAD risk, and that this polymorphism has interactions with gender and smoking. Our findings may be helpful in identifying individuals who are at increased risk for developing CAD.

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