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IL-8 -251A/T polymorphism contributes to coronary artery disease susceptibility in a Chinese population

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ABSTRACT. Interleukin-8 (IL-8) is a mediator of inflammation and plays an important role in regulating immune responses. To date, several studies have tested the association between *IL-8* gene polymorphisms and development of coronary artery disease (CAD), but their results have proved to be inconsistent. We conducted an investigation to assess the relationship between the *IL-8* -251A/T (rs4073) sequence variant and CAD in a Chinese population. Between April 2013 and January 2015, 217 patients with coronary angiography-confirmed CAD were enrolled in our study, along with 245 control subjects. *IL-8* -251A/T genotyping was performed using a polymerase chain reaction-restriction fragment length polymorphism assay. A chi-square test revealed that *IL-8* -251A/T genotype distributions significantly differed between CAD patients and control subjects (chi-square = 8.29, P < 0.02). Moreover, multiple-logistic regression analysis showed that individuals carrying TA [odds ratio (OR) = 1.59, 95% confidence interval (CI) = 1.01-2.57] and AA

(OR = 2.06, 95%CI = 1.21-3.52) genotypes were at increased risk of CAD compared to those with the TT genotype. Under dominant (OR = 1.75, 95%CI = 1.13-2.73) and recessive (OR = 1.54, 95%CI = 1.02-2.37) genetic models, the *IL-8* -251A/T polymorphism also significantly correlated with CAD. In conclusion, our results suggest that this variant is an independent risk factor for CAD development under codominant, dominant, and recessive models.

Key words: *IL-8* -251A/T; rs4073; Coronary artery disease; Polymorphism

INTRODUCTION

Cardiovascular disease is associated with high mortality and morbidity worldwide, and its incidence has been increasing in China recently (Ding et al., 2015). Coronary artery disease (CAD), caused by atherosclerosis, is the most common type of cardiovascular disease. The etiology of CAD is a long-term process involving many lifestyle factors and their interactions, including diet, hypertension, hypercholesterolemia, diabetes, obesity, and lack of physical activity (Campbell et al., 1998; Lindahl et al., 2000; Erbel and Görge, 2014; Bosevski and Lazarova-Trajkovska, 2015). However, not all individuals exposed to such risks develop CAD; therefore, it is thought that hereditary factors contribute to the pathology of this disease.

The *IL-8* gene is located on chromosome 4q13-q21, and comprises four exons, three introns, and a proximal promoter region (Mukaida et al., 1989). IL-8 plays an important role in regulating both inflammatory and immune processes (Modi et al., 1990; Gura, 1996). To date, several studies have examined the association between *IL-8* gene polymorphisms and risk of CAD and myocardial infarction, but with conflicting results (Vogiatzi et al., 2008; Ren and She, 2015; Wang et al., 2015). Therefore, we conducted the current investigation to assess the relationship between the *IL-8* -251A/T (rs4073) sequence variation and development of CAD in a Chinese population.

MATERIAL AND METHODS

Subjects

Between April 2013 and January 2015, 217 CAD patients were recruited from the First Department of Cardiovascular Internal Medicine of Zhengzhou People's Hospital and the People's Hospital of Linyi City. All patient diagnoses were confirmed by coronary angiography. Over the same period, 245 control subjects were randomly selected among individuals attending our hospital for a regular health examination. Using coronary angiography, all participants in this group were confirmed to be free of cardiovascular diseases, including CAD. Patients and controls with serious infectious diseases, severe heart failure, malignant tumors, dilated or hypertrophic cardiomyopathy, or serious kidney or liver diseases were excluded. Lifestyle habits of study participants and certain clinical data were ascertained using a self-designed questionnaire concerning gender, age, family history of CAD, tobacco smoking, alcohol consumption, body mass index (BMI), hypertension, and diabetes. Serum biochemical data were collected from medical records, and comprised levels of total cholesterol (TC),

triglycerides (TGs), and high- (HDL-C) and low-density lipoprotein cholesterol (LDL-C).

This study was performed with the permission of the Institutional Review Board of Zhengzhou People's Hospital and the People's Hospital of Linyi City. Written informed consent was obtained from all subjects prior to participation.

Genotyping

A blood sample (5 mL) was obtained from each participant and stored at -20°C until use. Isolation of genomic DNA was carried out using a TIANamp blood DNA kit (Tiangen, Beijing, China), and genotyping of *IL-8* -251A/T was performed with a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay. Primer Express 2.0 (Applied Biosystems, Foster City, CA, USA) was used to design the following PCR primers targeting the *IL-8* region containing the -251A/T variation: forward, 5'-TGC CCC TTC ACT CTG TTA AC-3'; and reverse, 5'-GAA GTC CCA CAA TTT GGT G-3'. PCRs were carried out under the following conditions: denaturation at 95°C for 5 min, then 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s, before a final extension at 72°C for 7 min. PCR products were kept at 4°C until needed. Amplified fragments of the region containing the *IL-8* -251A/T polymorphism were subsequently digested with the restriction enzyme *Mfe*I. Digestion products were visualized under ultraviolet light.

Statistical analysis

Chi-square tests were carried out to assess differences between CAD patients and control subjects with respect to lifestyle and clinical characteristics, including serum measurements. Hardy-Weinberg equilibrium (HWE) was determined by the chi-square test. Multivariate logistic regression analyses were also conducted, in which single-putative risk factors were analyzed to determine crude odds ratios (ORs) and 95% confidence intervals (CIs). Multiple-logistic regression analysis was carried out to adjust for potential confounding factors. All statistical analyses were achieved using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). P values <0.05 were considered statistically significant.

RESULTS

The lifestyle and clinical characteristics of patients and control subjects are presented in Table 1. The two groups were comparable in terms of age (chi-square = 3.10, P = 0.08), alcohol consumption (chi-square = 3.55, P = 0.06), and family history of CAD (chi-square = 0.54, P = 0.46). Using chi-square tests, significant differences were observed between CAD patients and control subjects with respect to gender (chi-square = 4.80, P = 0.03), BMI (chi-square = 16.35, P < 0.01), hypertension (chi-square = 27.57, P < 0.01), diabetes (chi-square = 5.16, P = 0.02), tobacco smoking (chi-square = 9.66, P = 0.002), and levels of TC (chi-square = 6.96, P < 0.05), TG (chi-square = 4.33, P < 0.05), HDL-C (chi-square = 2.36, P = 0.01), and LDL-C (chi-square = 6.58, P < 0.05).

Distributions of *IL-8* -251A/T genotypes are shown in Table 2. In total, 47 (21.66%), 101 (46.54%), and 69 (31.80%) CAD patients, and 80 (32.65%), 108 (44.08%), and 57 (23.27%) control subjects carried TT, TA, and AA genotypes, respectively. The chi-square test revealed a statistically significant difference in genotype distributions between patients

and controls (chi-square = 8.29, P = 0.02). Moreover, *IL-8* -251A/T genotype frequencies were consistent with HWE, with P values of 0.38 and 0.08 for the CAD and control groups, respectively.

Table 1. Clinical and lifestyle characteristics of study subjects.

Characteristic	Patients (N = 217)	%	Controls (N = 245)	%	Chi-square	P
Gender						
Female	74	34.10	108	44.08		
Male	143	65.90	137	55.92	4.80	0.03
Age (years)						
<50	129	59.45	165	67.35		
≥50	88	40.55	80	32.65	3.10	0.08
BMI (kg/m^2)						
<24	84	38.71	141	57.55		
≥24	133	61.29	104	42.45	16.35	<0.01
Hypertension						
No	103	47.47	175	71.43		
Yes	114	52.53	70	28.57	27.57	<0.01
Diabetes						
No	178	82.03	219	89.39		
Yes	39	17.97	26	10.61	5.16	0.02
Tobacco smoking						
No	125	57.60	175	71.43		
Yes	92	42.40	70	28.57	9.66	0.002
Alcohol consumption						
No	140	64.52	178	72.65		
Yes	77	35.48	67	27.35	3.55	0.06
Family history of CAD						
No	206	94.93	236	96.33		
Yes	11	5.07	9	3.67	0.54	0.46
TC		195.35 ± 32.42		174.62 ± 31.56	6.96	<0.05
TG		132.54 ± 38.42		118.36 ± 31.90	4.33	<0.05
HDL-C		40.23 ± 17.42		43.45 ± 11.56	2.36	0.01
LDL-C		117.72 ± 20.45		104.52 ± 22.42	6.58	<0.05

BMI = body mass index, CAD = coronary artery disease, TC = total cholesterol, TG = triglycerides, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol.

Table 2. Distribution of *IL-8* -251A/T genotypes in coronary artery disease patients and control subjects.

Genotype	Patients (N = 217)	%	Controls (N = 245)	%	Chi-square	P	P for HWE	
							Patients	Controls
TT	47	21.66	80	32.65				
TA	101	46.54	108	44.08				
AA	69	31.80	57	23.27	8.29	0.02	0.38	0.08

HWE = Hardy-Weinberg equilibrium.

Multiple-logistic regression analysis showed that individuals carrying the TA (OR = 1.59, 95%CI = 1.01-2.57) and AA (OR = 2.06, 95%CI = 1.21-3.52) genotypes of *IL-8* -251A/T were at increased risk of CAD compared to those with the TT genotype (Table 3). Under a dominant model, the TA+AA genotype showed a statistically significant correlation with CAD compared to the TT genotype (OR = 1.75, 95%CI = 1.13-2.73), whereas under a recessive model, the AA genotype was significantly associated with CAD risk compared to the TT+TA genotype (OR = 1.54, 95%CI = 1.02-2.37).

Table 3. Association between the *IL-8* -251A/T genetic polymorphism and development of coronary artery disease assessed by multiple-logistic regression analysis.

Model and genotype	Patients (N = 217)	%	Controls (N = 245)	%	OR (95%CI)[1]	P
Codominant						
TT	47	21.66	80	32.65	1.0 (Ref.)	-
TA	101	46.54	108	44.08	1.59 (1.01-2.57)	0.04
AA	69	31.8	57	23.27	2.06 (1.21-3.52)	0.005
Dominant						
TT	47	21.66	80	32.65	1.0 (Ref.)	-
TA+AA	170	78.34	165	67.35	1.75 (1.13-2.73)	0.008
Recessive						
TT+TA	148	68.2	188	76.73	1.0 (Ref.)	-
AA	69	31.8	57	23.27	1.54 (1.02-2.37)	0.04

[1]Adjusted for gender, age, body mass index, hypertension, diabetes, tobacco smoking, total cholesterol, triglycerides, and high- and low-density lipoprotein cholesterol. OR = odds ratio, CI = confidence interval, Ref. = reference.

DISCUSSION

Here, we report the results of a case-control study investigating the association between the *IL-8* -251A/T polymorphism and CAD risk, in which this variant was found to correlate with this disease under codominant, dominant, and recessive models.

IL-8 promotes the production of other cytokines. IL-1, TNF-α, LPS, and phorbol myristate acetate can induce the synthesis and secretion of IL-8 by monocytes, macrophages, fibroblasts, and endothelial cells (Hébert et al., 1990; Mielke et al., 1990; Strieter et al., 1990). Rus et al. (1996) reported that IL-8 expression can be induced by complement activation, and that this may contribute to the increased IL-8 levels found in atherosclerotic vessel walls. Moreover, this chemokine is involved in inflammation during the initiation and progression of atherosclerosis. IL-8 production remains elevated for an extended period during acute inflammation, whereas other inflammatory cytokines are cleared within a few hours (DeForge et al., 1992; Shebuski and Kilgore, 2002). Liu et al. (1997) suggested that IL-8 contributes to the recruitment of T lymphocytes and smooth muscle cells to the subendothelial space and has a major role in the formation of atherosclerotic lesions, whereas Nair et al. (2014) showed that *IL-8* gene expression is a predictor of cardiovascular risk. Therefore, IL-8 may play a role in the pathogenesis of atherosclerosis, and thus appears to be associated with the development of CAD.

Previous studies have demonstrated that sequence variations in this gene are associated with CAD, but their results have been inconsistent (Vogiatzi et al., 2008; He et al., 2014; Ren and She, 2015; Yang et al., 2015). Vogiatzi et al. (2008) conducted an investigation into the influence of common *IL-8* polymorphisms on CAD risk, showing a combination of the -251A/T and -781C/T variants to be associated with susceptibility to this disease. However, He et al. (2014) and Ren and She (2015) failed to establish a link between the -251A/T polymorphism and CAD risk, and a similar result was obtained by Yang et al. (2015) in a study of several IL gene variants. In the present study, we found this same polymorphism to be independently associated with increased risk of CAD. Such discrepancies may be the result of differences in study populations, selection of patients and control subjects, or sample sizes.

In conclusion, our findings suggest that the *IL-8* -251A/T polymorphism constitutes an independent risk factor for the development of CAD under codominant, dominant, and recessive models. Further studies with large sample sizes including subjects of various ethnicities are greatly needed to confirm our results.

Conflicts of interest

The authors declare no conflict of interest.

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