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Association between *IL-10*-G1082A polymorphisms and the development of coronary artery disease in a Chinese population

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ABSTRACT. We conducted a case-control study to investigate the association between *IL-10*-G1082A (rs1800896) polymorphism and the development of coronary artery disease (CAD) in the Chinese population. We recruited 295 CAD patients and 355 healthy controls from the Fifth Affiliated Hospital of Zhengzhou University between April 2012 and December 2014. Subjects were genotyped for *IL-10*-G1082A polymorphisms by using polymerase chain reaction-restriction fragment length polymorphism. We observed significant differences in the genotype frequencies of GG, AG, and AA between CAD patients and controls (χ^2 = 17.38, P < 0.001). Multivariate logistic regression analyses revealed that individuals with the AA genotype at *IL-10*-1082A/G was associated with increased risk for CAD as compared to those with the GG genotype (OR = 2.96, 95%CI = 1.70-5.23, P < 0.001). Our results indicated that *IL-10* -1082A/G polymorphism was associated with susceptibility to CAD in both dominant (OR = 1.59,

95%CI = 1.15-2.20, P = 0.004) and recessive (OR = 2.58, 95%CI = 1.53-4.45, P < 0.001) models. In conclusion, our study suggests that *IL-10*-1082A/G polymorphism is associated with an increased risk for CAD.

Key words: *IL-10*-1082A/G; Polymorphism; Coronary artery disease

INTRODUCTION

Cardiovascular disease is a complex and serious disease, associated with high morbidity and mortality worldwide (He et al., 2005; Go et al., 2014). Coronary artery disease (CAD) is one of the most common cardiovascular diseases. It is well known that CAD arises due to multiple lifestyle and environmental factors such as hypertension, diabetes, tobacco smoking and family history of hypertension (Jayashree et al., 2015; Chhabra et al., 2016). However, detailed etiology of this disease is not well understood. Moreover, it is reported that genetic factors also contribute to the development of CAD. Previous studies have reported that several genetic factors such as monocyte chemoattractant protein-1 (MCP-1), fatty acid desaturase 1/2 (FADS1/FADS2), cyclooxygenase-2 (COX-2), and matrix metalloproteinase-2 (MMP-2) play important roles in the development of CAD (Angeles-Martínez et al., 2015; Shi et al., 2015a; Yang et al., 2015; Zhang et al., 2015).

Previous studies reported that cytokines play an important role in the migration of neutrophils, lymphocytes, as well as antigen-presenting cells, and thus contribute to the inflammatory response elicited during CAD development (Sun et al., 2014; Oikonomou et al., 2014; Shi et al., 2015b). Previous studies have associated *IL-10*-G1082A polymorphism with risk for CAD; however, the results were inconclusive (Karaca et al., 2011; Guo et al., 2012; Chao et al., 2014; Elsaid et al., 2014; Lin et al., 2014; Ren and She, 2015). Therefore, we conducted a case-control study to investigate the association between *IL-10*-G1082A (rs1800896) polymorphism and development of CAD in the Chinese population.

MATERIAL AND METHODS

Patients

CAD patients (295) were recruited from the Fifth Affiliated Hospital of Zhengzhou University between April 2012 and December 2014. All patients with CAD underwent coronary angiography according to standardized protocols and were independently diagnosed by two cardiologists. CAD patients had a history of a myocardial bridge, serious kidney or liver diseases, as well as malignant tumors were excluded from our study.

Healthy controls (355) were randomly selected from individuals who underwent health examinations and coronary angiography. All control subjects were confirmed to be free of CAD, and had no history of arteriosclerotic lesions. Control subjects who had a myocardial bridge, congenital heart diseases, childhood hypertension, serious kidney or liver diseases, as well as cancers were also excluded from this study.

The demographic characteristics of all study subjects were collected from structured questionnaires, which included information regarding sex, age, alcohol consumption, and tobacco smoking. Clinical information was collected from medical records, which included TC, LDL-c, HDL-c, and TG levels.

Blood sample (5 mL) was obtained from each patient and control for analysis, and the written informed consent form was signed by all study subjects prior to their participation in the study. The study protocol was approved by the Ethics Committee of the Fifth Affiliated Hospital of Zhengzhou University.

DNA extraction and SNP genotyping

DNA was extracted from peripheral blood leukocytes by using a TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer instructions. *IL-10*-G1082A polymorphism was genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Forward and reverse primers of *IL-10*-G1082A for PCR were as follows: 5'-CCAAGACAACTAAGGCTCCTTT-3' and 5'-GCTTCTTATATGCTAGTCAGGTA-3'. Amplified gene product was digested with the restriction enzyme *Mnl*I, and the length of the digested fragment for *IL-10*-G1082A was 139 bp. The length of the digested fragment for the A allele was 139 bp, while that for those of the G allele was 106 and 33 bp. The reaction conditions for PCR were as follows: one cycle of initial DNA denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min; and final extension was carried out at 72°C for 5 min. The PCR products were analyzed by electrophoresis on a 2% agarose gel and stained with ethidium bromide. The DNA bands were visualized under UV light.

Statistical analysis

The demographic and clinical characteristics, as well as *IL-10*-G1082A genotype frequencies in patients with CAD and control subjects were compared using chi-squared (χ^2) tests or Student's *t*-test. The goodness-of-fit χ^2-test was carried out in all patient and control samples to determine whether the genotype frequencies at *IL-10* -G1082A deviated from the Hardy-Weinberg equilibrium (HWE). Unconditional logistic regression analysis was conducted to assess the role of *IL-10*-G1082A in the development of CAD, and the results were analyzed by OR and 95%CI. Statistical analysis was conducted using the SPSS 16.0 software, and $P < 0.05$ was considered statistically significant.

RESULTS

The distributions of demographic and clinical characteristics of study subjects are summarized in Table 1. The mean ages of CAD patients and controls were 58.65 ± 11.62 and 57.53 ± 12.15 years, respectively. We did not find any significant differences between CAD patients and controls in terms of age ($\chi^2 = 1.19$, $P = 0.12$), sex ($\chi^2 = 0.007$, $P = 0.94$), or alcohol consumption ($\chi^2 = 3.18$, $P = 0.07$). As compared to the control group, we found that patients with CAD had a habit of tobacco smoking ($\chi^2 = 61.13$, $P < 0.001$), higher TC ($\chi^2 = 7.17$, $P < 0.001$), LDL-c ($\chi^2 = 4.79$, $P < 0.001$), and TG levels ($\chi^2 = 10.37$, $P < 0.001$), but lower HDL-c levels ($\chi^2 = 5.17$, $P < 0.001$).

We also observed significant differences in the genotype frequencies of GG, AG, and AA between CAD patients and controls ($\chi^2 = 17.38$, $P < 0.001$) (Table 2). The genotypic distributions of *IL-10*-1082A/G in the CAD patients and control subjects did not deviate from the Hardy-Weinberg equilibrium, and the P values were 0.08 and 0.78, respectively. Moreover,

the calculated minor allele frequencies of *IL-10*-1082A/G in controls were similar to those obtained from the National Center for Biotechnology Information SNP database.

Multivariate logistic regression analyses revealed that individuals with the AA genotype at *IL-10*-1082A/G were at increased risk for CAD as compared to those with the GG genotype (OR = 2.96, 95%CI = 1.70-5.23, P < 0.001) (Table 3). In the dominant model, compared to the GG genotype, the AG + AA genotype was associated with an elevated risk for CAD (OR =1.59, 95%CI = 1.15-2.20, P = 0.004). In addition, the AA genotype was associated with increased risk of CAD as compared with the GG + AG genotype in a recessive model (OR = 2.58, 95%CI = 1.53-4.45).

Table 1. Demographic and clinical characteristics of study subjects.

	CAD patients (N = 295)	%	Controls (N = 355)	%	χ^2 test or *t*-test	P value
Mean age (years)	58.65 ± 11.62		57.53 ± 12.15		1.19	0.12
Gender						
Male	226	76.61	271	76.34		
Female	69	23.39	84	23.66	0.007	0.94
Alcohol consumption						
Never	143	48.47	197	55.49		
Current or ever	152	51.53	158	44.51	3.18	0.07
Tobacco smoking						
Never	125	42.37	258	72.68		
Current or ever	170	57.63	97	27.45	61.13	<0.001
TC (mM)	188.22 ± 34.13		169.30 ± 32.94		7.17	<0.001
LDL-c (mM)	114.21 ± 24.53		105.32 ± 22.74		4.79	<0.001
HDL-c (mM)	38.47 ± 18.10		44.52 ± 11.47		5.17	<0.001
TG (mM)	142.91 ± 31.58		119.47 ± 26.04		10.37	<0.001

Table 2. Genotype distributions of *IL-10*-1082A/Gin CAD patients and healthy control subjects.

IL-10-1082A/G	Patients (N = 295)	%	Controls (N = 355)	%	P for HWE		χ^2-test	P value	Minor allele frequency	
					In cases	In controls			In database	In controls
rs1800896										
GG	120	40.68	185	52.11						
AG	125	42.37	144	40.56						
AA	50	16.95	26	7.32	0.08	0.78	17.38	<0.001	0.2722	0.2761

Table 3. Association between *IL-10*-1082A/G polymorphism and the risk of CAD.

IL-10-1082A/G	Patients (N = 295)	%	Controls (N = 355)	%	OR (95%CI)[1]	P value
Co-dominant						
GG	120	40.68	185	52.11	Reference group	-
AG	125	42.37	144	40.56	1.34 (0.95-1.89)	0.09
AA	50	16.95	26	7.32	2.96 (1.70-5.23)	<0.001
Dominant						
GG	120	40.68	185	52.11	Reference group	-
AG+AA	175	59.32	170	47.89	1.59 (1.15-2.20)	0.004
Recessive						
GG+AG	245	83.05	329	92.68	Reference group	-
AA	50	16.95	26	7.32	2.58 (1.53-4.45)	<0.001

[1]Adjusted for gender, age, tobacco smoking, TC, LDL-c, HDL-c, and TG.

DISCUSSION

In this hospital-based case-control study, we assessed the role of *IL-10*-1082A/G gene polymorphism (rs1800896) in the development of CAD. Our results indicated that *IL-10*-1082A/G polymorphism is associated with an increased risk of CAD.

Previous studies have reported that *IL-10*-1082A/G polymorphism was associated with various diseases such as Graves' disease, liver cirrhosis, ischemic stroke, deep venous thrombosis, and atopic asthma (Jin et al., 2014; Tang et al., 2014; Zheng et al., 2014; Guo et al., 2015; Liang et al., 2015). Zheng et al. (2014) conducted a meta-analysis with 4,716 asthmatic patients and 5,093 controls, and reported that *IL-10* promoter-1028A/G polymorphism was associated with susceptibility to asthma. Tang et al. (2014) conducted a case-control study on patients with deep venous thrombosis, and reported that *IL-10*-1082A/G polymorphism was correlated with the development of deep venous thrombosis. A meta-analysis by Jin et al. (2014), which consisted of seven independent studies, showed that *IL-10*-1082A/G polymorphism was associated with ischemic stroke in Asians. Guo et al. (2015) conducted a meta-analysis consisting of 12 independent studies and reported that *IL-10*-1082A/G polymorphism did not contribute to HCV-related liver cirrhosis. In a case-control study by Liang et al. (2015), it was reported that *IL-10* -1082A/G polymorphism was a predictive biomarker for Graves' diseases.

Several previous studies reported an association between *IL-10*-1082A/G polymorphism and the development of coronary artery diseases; however, the results were inconclusive (Guo et al., 2012; Elsaid et al., 2014; Li et al., 2015; Ren and She, 2015). Li et al. (2015) conducted a case-control study in the Chinese population and did not find any association between *IL-10*-1082A/G polymorphism and the risk of coronary artery diseases. Similarly, Guo et al. (2012) reported that *IL-10*-1082A/G polymorphism is unlikely to be associated with CAD. In contrast, a study by Ren and She (2015) with 325 CAD patients and 342 controls reported that *IL-10*-1082A/G polymorphism contributes to the risk for CAD, especially in individuals with hypertension or diabetes mellitus, as well as in smokers. This was confirmed in a study by Elsaid et al. (2014), which suggested that *IL-10*-1082A/G polymorphism is associated with an increased susceptibility to CAD. Two previous meta-analyses have also reported that the GA + AA genotypes were associated with an increased risk for CAD and atherosclerosis (Wang et al., 2012; Chao et al., 2014). In our study, we found that the AA and AG + AA genotypes were associated with the development of CAD. Further studies are needed to confirm our findings.

In conclusion, our results suggest that *IL-10*-1082A/G polymorphism is associated with an increased risk for CAD. Future studies conducted in patients of different ethnic backgrounds and in a larger cohort may help elucidate the impact of these polymorphisms on the development of CAD.

Conflicts of interest

The authors declare no conflicts of interest.

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