Role of interleukin-10 gene polymorphisms in the development of coronary artery disease in Chinese population

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ABSTRACT. The aim of this study was to investigate the association between three common SNPs (-1082A/G, -819T/C, and -592A/C) in the interleukin 10 (*IL-10*) gene, and the development of coronary artery disease. Between January 2013 and December 2014, 272 patients with coronary artery disease and control subjects (each) were recruited for this study from the Huaihe Hospital of Henan University. The *IL-10*-1082A/G, -819T/C and -592A/C gene polymorphisms were analyzed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. Logistic regression analyses revealed an association between the AA and GA+AA genotypes of *IL-10*-1082G/A and an elevated risk of coronary artery disease, compared to the GG genotype [adjusted odds ratio (OR) = 2.31 and 1.49; 95% confidence interval (CI) = 1.29-4.19 and 1.04-2.12, respectively]. The AG+GG genotype was associated with a moderately increased risk of coronary artery disease in smokers (adjusted OR = 2.74; 95% CI = 1.01-3.01). In conclusion, the AA and GA+AA genotypes of *IL-10*-1082G/A were associated with an elevated risk of coronary artery disease; the *IL-10*-1082G/A gene polymorphism

also interacted with the tobacco smoking habits, contributing to the development of coronary artery disease.

Key words: Interleukin-10; Coronary artery disease; Polymorphism

INTRODUCTION

Coronary artery disease remains the leading cause of mortality and morbidity worldwide; this disease is also the leading cause of death in China. The development of coronary artery disease is a complex process, including environmental and genetic factors. Many environmental factors have been reported to play an important role in the development of coronary artery disease, such as alcohol consumption, tobacco usage, higher body mass index (BMI), dyslipidemia, hypertension, and diabetes mellitus (Sun et al., 2014; Puaschitz et al., 2015; van Setten et al., 2015). Some studies have also reported that variations in genes, such as aldehyde dehydrogenase 2, *GP78*, *CYP17A1*, interleukin-6 (*IL*-6), and *IL-17*, as well *MMP*-1, contribute to the development of coronary artery disease (Zhang et al., 2011; Cha et al., 2014; Qintao et al., 2014; Xu et al., 2014; Dai et al., 2015; Wang et al., 2015).

A multitude of inflammatory markers have been reported to play an important in the development of atherogenesis (Maier et al., 2005; Rosenson, 2008). Interleukins are important factors that cause a chronic vascular inflammatory response to atherosclerosis (Aukrust et al., 2008; Tziakas et al., 2008). The encoding gene of IL-10 is located on chromosome 1 at the 1q31-1q32 junction. IL-10 is an anti-inflammatory T-cell cytokine. Experimental studies have reported that IL-10 is a pro-inflammatory cytokine involved in both innate and acquired immune responses, which plays a critical role in the development of atherosclerosis (Cai et al., 2014; Chao et al., 2014; Yu et al., 2015). In this study, we investigated the possible association between three common SNPs (-1082A/G, -819T/C, and -592A/C) in the *IL-10* gene and the development of coronary artery disease.

MATERIAL AND METHODS

Study subjects

Patients with proven coronary artery disease (n = 306) were recruited from the Huaihe Hospital of Henan University between January 2013 and December 2014. Coronary artery disease was diagnosed in patients when a diameter stenosis of 50% was observed in any of the main coronary arteries. Patients with a history of prior angioplasty, coronary artery bypass surgery, or myocardial infarction history were also included as coronary artery disease subjects. Subjects were excluded based on the following criteria: presence of congenital heart disease, peripheral arterial disease, any type of autoimmune disease, renal and liver disease, or cancers. Finally, 272 patients with coronary artery disease were enrolled to our study, at a participation rate of 88.89%.

Two hundred and seventy two control subjects free of coronary artery disease were selected from among individuals who underwent a regular health examination at the hospital clinic between January 2013 and December 2014. The control subjects were age- and gender-matched with the patients. Cases and controls were interviewed using a standardized questionnaire, which included questions regarding their socio-demographic characteristics (age, sex, alcohol consumption, tobacco smoking, and body mass index). Detailed clinical data, including those on hypertension, diabetes mellitus, and total cholesterol (TC), triglyceride (TG), low density

lipopolysaccharide cholesterol (LDL-c), and high density lipopolysaccharide cholesterol (HDL-c) levels, of patients with coronary artery disease was collected from their medical records. Blood samples (5 mL) and signed informed consent forms were obtained from each patient and control subject prior to their participation in the study. The study protocol was approved by the Clinical Research Ethics Committee of the Huaihe Hospital of Henan University.

Genotyping

The blood samples of patients with coronary artery disease and control subjects were collected in ethylene diamine tetra-acetic acid (EDTA)-coated tubes, and stored at -20°C until use. The *IL-10* -1082A/G, -819T/C, and -592A/C gene polymorphisms were analyzed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The forward and reverse primers used for this purpose were as follows: *IL-10*-1082A/G, 5'-TCATTCTATGTGCTGGAGATGG-3' and 5'-TGGGGGAAGTGGGTAAGAGT-3'; *IL-10*-819T/C, 5'-GGTGAGCACTACCTGACTAGC-3' and 5'-CCTAGGTCACAGTGACGTGG-3'; and *IL-10*-592A/C, 5'-CCTGAGCACTAGGTGACTAGC-3' and 5'-GGTACCTCACAGTGACGTCC-3', respectively. The samples were amplified using the following cycling program: one cycle of 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, and extension at 72°C for 2 min; and a final extension at 72°C for 5 min. Quality control was ensured by blind genotyping of the subject samples. The PCR products were verified by a 2% agarose gel electrophoresis, staining with ethidium bromide, and ultraviolet light analysis in order.

Statistical analysis

Differences in the distributions of demographic and clinical characteristics between patients with coronary artery disease and control subjects were compared by the χ^2- or *t*-test. The distribution of genotypes in the controls was tested for deviation from the Hardy-Weinberg equilibrium (HWE) using the goodness-of-fit χ^2-test. The association between *IL-10*-1082A/G, -819T/C, and -592A/C polymorphisms and development of coronary artery disease was determined by estimating the odds ratio (OR) and 95% confidence interval (95% CI) by logistic regression. The major homozygous genotype of the *IL-10*-1082A/G, -819T/C, and -592A/C polymorphisms was used as a reference. The samples were statistically analyzed using the SPSS 21.0 package (SPSS Inc., Chicago, IL, USA). A P value < 0.05 was considered to indicate a significant difference.

RESULTS

The demographic and clinical characteristics of patients with coronary artery disease and control subjects are summarized in Table 1. No significant difference was found between the patients and control subjects in terms of sex (χ^2 = 0.00, P = 1.00) and age (χ^2 = 0.36, P = 0.55). Patients with coronary artery disease were more likely to have a higher BMI (*t* = 6.99, P < 0.001), consume alcohol (χ^2 = 4.61, P = 0.03), smoke tobacco (χ^2 = 58.26, P < 0.001), and be diagnosed with hypertension (χ^2 = 16.66, P < 0.001) and/or diabetes mellitus (χ^2 = 21.51, P < 0.001), compared to the control subjects. In addition, patients with coronary artery disease showed higher levels of TC (*t* = 2.35, P = 0.01), LDL-c (*t* = 3.70, P < 0.001), HDL-c (*t* = 3.40, P < 0.001), and TG (*t* = 3.40, P < 0.001), compared to the subjects in the control group.

Table 1. Demographic and clinical characteristics of patients with coronary artery disease and control subjects.

Variables	Patients (%)	Controls (%)	χ^2-test or *t*-test	P value
Mean age, year				
<60	152 (55.88)	145 (53.31)		
≥60	120 (44.12)	127 (46.69)	0.36	0.55
Gender				
Females	202 (74.26)	202 (74.26)		
Males	70 (25.74)	70 (25.74)	0.00	1.00
Body mass index, kg/m^2	26.4 ± 4.7	23.7 ± 4.3	6.99	< 0.001
Alcohol drinking				
Never	115 (42.28)	140 (51.47)		
Ever	157 (57.72)	132 (48.53)	4.61	0.03
Tobacco smoking				
Never	94 (34.56)	183 (67.28)		
Ever	178 (65.44)	89 (32.72)	58.26	< 0.001
Hypertension				
No	148 (54.41)	194 (71.32)		
Yes	124 (45.59)	78 (28.68)	16.66	< 0.001
Diabetes mellitus				
No	197 (72.43)	240 (88.24)		
Yes	75 (27.57)	32 (11.76)	21.51	< 0.001
TC, mM	4.73 ± 1.24	4.50 ± 1.03	2.35	0.01
LDL-c, mM	2.44 ± 0.62	2.26 ± 0.51	3.70	< 0.001
HDL-c, mM	1.27 ± 0.32	1.19 ± 0.22	3.40	< 0.001
TG, mM	2.43 ± 1.06	2.06 ± 0.98	4.23	< 0.001

TC = total cholesterol; LDL-c = low density lipopolysaccharide cholesterol; HDL-c = high density lipopolysaccharide cholesterol; TG = triglyceride.

The genotype distributions of IL-10-1082A/G, -819T/C and -592A/C in the control group conformed to the Hardy-Weinberg equilibrium (HWE), and the *P* values (for HWE) were 0.95, 0.06, and 0.63, respectively (Table 2). We observed significant differences in the genotype distributions of *IL-10*-1082A/G (χ^2 = 9.39, P = 0.009); however, IL-10-819T/C and -592A/C exhibited no such differences. Logistic regression analysis helped associate the AA and GA+AA genotypes of *IL-10*-1082G/A with an elevated risk of coronary artery disease, compared to the GG genotype (adjusted OR = 2.31 and 1.49; 95% CI = 1.29-4.19 and 1.04-2.12, respectively). However, the *IL-10*-819T/C and -592A/C gene polymorphisms were not significantly associated with increased risk of coronary artery disease.

Table 2. Association between the *IL-10*-1082A/G, -819T/C, and -592A/C gene polymorphisms and the development of coronary artery disease.

IL-10 gene	Patients	Controls	P for HWE	OR (95%CI)[1]	P value
-1082G/A					
GG	105 (38.60)	132 (48.53)		1.0 (Ref.)	-
GA	121 (44.49)	116 (42.65)		1.31 (0.90 - 1.91)	0.14
AA	46 (16.91)	25 (8.82)	0.95	2.31 (1.29 - 4.19)	0.003
GA + AA	167 (61.40)	141 (51.47)		1.49 (1.04 - 2.12)	0.02
-819T/C					
TT	110 (40.44)	118 (43.38)		1.0 (Ref.)	-
TC	113 (41.54)	111 (40.81)		1.09 (0.74 - 1.61)	0.64
CC	49 (18.01)	43 (15.81)	0.06	1.22 (0.73 - 2.05)	0.42
TC + CC	162 (59.56)	154 (56.62)		1.13 (0.79 - 1.61)	0.49
-592A/C					
AA	100 (36.76)	110 (40.44)		1.0 (Ref.)	-
AC	128 (47.06)	123 (45.22)		1.14 (0.78 - 1.68)	0.47
CC	44 (16.18)	39 (14.34)	0.63	1.24 (0.72 - 2.13)	0.41
AC + CC	172 (63.24)	162 (59.56)		1.17 (0.81 - 1.67)	0.38

[1]Adjusted for sex, age, body mass index (BMI), alcohol drinking, tobacco smoking, hypertension, and diabetes mellitus. HWE = Hardy-Weinberg equilibrium; OR = odds ratio; CI = confidence interval.

The association between *IL-10*-1082G/A gene polymorphisms and the development of coronary artery disease was stratified based on alcohol consumption, tobacco smoking, and a diagnosis of hypertension and/or diabetes mellitus (Table 3). The AG+GG genotype was found to be associated with moderately increased risk of coronary artery disease in smokers (adjusted OR - 2.74; 95% CI = 1.01-3.01).

Table 3. Analysis of the *IL-10*-1082G/A gene polymorphism stratified based on demographic and clinical characteristics.

Variables	Patients		Controls		OR (95%CI)	P value
	AA	AG + GG	AA	AG + GG		
Alcohol drinking						
Never	47	68	69	71	1.41 (0.83 - 2.39)	0.18
Ever	58	99	63	69	1.56 (0.94 - 2.56)	0.06
Tobacco smoking						
Never	41	53	88	95	1.20 (0.70 - 2.04)	0.48
Ever	64	114	44	45	2.74 (1.01 - 3.01)	0.03
Hypertension						
No	58	90	94	100	1.46 (0.92 - 2.31)	0.09
Yes	47	77	38	40	1.56 (0.84 - 2.87)	0.13
Diabetes mellitus						
No	78	119	115	125	1.40 (0.94 - 2.09)	0.08
Yes	27	48	17	15	2.01 (0.80 - 5.08)	0.1

OR = odds ratio; CI = confidence interval.

DISCUSSION

Genetic polymorphisms in functional cytokines could change their expression, thereby influencing the development of vascular lesions (Weng et al., 2010; Khankhanian et al., 2013). IL-10 is produced by T-lymphocytes, and is an important anti-inflammatory and immune-suppressive cytokine that regulates angiogenesis in many inflammation-related diseases (Bantis et al., 2008; Javor et al., 2014; Peng et al., 2015; Yu et al., 2015). The *IL-10* gene is a candidate gene in the pathophysiological mechanism of auto-immune/inflammatory disease, as it can regulate both the cellular and humoral immunity. In this study, the AA and GA+AA genotypes of the *IL-10*-1082G/A polymorphism were found to be associated with an elevated risk of coronary artery disease; in addition, the *IL-10*-1082G/A polymorphism associated with tobacco smoking in the development of coronary artery disease.

Previous studies have reported an association between polymorphisms in the *IL-10* gene and the development of cardiovascular disease (Karaca et al., 2011; Yu et al., 2012; Jin et al., 2013; Elsaid et al., 2014; Lin et al., 2014); however, the results of these studies have been inconsistent. Yu et al. (2012) discovered a significant association between the SNPs at positions -592C/A and -819C/T and susceptibility to ischemic heart disease in a Korean population. Jin et al. (2013), in a study comprising 249 patients and 132 unaffected controls selected from a Chinese population, reported an association between the *IL-10*-592A/C polymorphism and increased risk of coronary heart disease. On the other hand, Elsaid et al. (2014), who conducted a case-control study with 108 Egyptian patients with coronary artery disease and 143 healthy subjects, reported that the G allele of *IL-10*-1082G/A was associated with an increased prevalence of coronary artery disease. Meanwhile, Lin et al. (2014), reported a correlation between polymorphisms in the *IL-10* gene (*IL-10*-1082G/A) and the development of coronary artery aneurysm in a Taiwanese population (Lin et al.,

2014). However, other studies have reported inconsistent results. Karaca et al. (2011) discovered an association between the *IL-10*-1082A/G polymorphism and the development of coronary heart disease, while *IL-10*-819T/C and -592A/C did not. A recent meta-analysis comprising 16 studies suggested that the *IL-10*-1082A/G polymorphism was associated with an increased risk of atherosclerosis (Chao et al., 2014). Wang et al. (2012), in a meta-analysis of 6 case-control studies conducted in a Caucasian population, suggested that the A allele of *IL-10*-1082A/G contributed to increased risk of coronary heart disease. In this study, the A allele of *IL-10*-1082G/A was correlated with increased risk of coronary artery disease. The discrepancies among the above results may be attributed to ethnic variations, differences in the source of patients and sample size, and to chance.

We also observed an association between the *IL-10*-1082A/G gene polymorphism and tobacco smoking habits in the development of coronary artery disease. Previous studies reported that tobacco smoking interacted with cytokine genes, contributing to the development of several diseases (Meisel et al., 2002; Kuo et al., 2014). Further studies are required to confirm our findings.

Our study includes several limitations. Firstly, patients and controls were selected from a single hospital, which suggested that the selected controls may not be representative of the general population. Secondly, the sample size was relatively small; this limited sample size could result in a lack of power, which could explain our failure in finding an association with the *IL-10* polymorphisms.

In conclusion, the results of our study suggested an association between the *IL-10*-1082A/G gene polymorphisms and an elevated risk of coronary artery disease; in addition, *IL-10*-1082G/A interacts with the tobacco smoking habits in contributing to the development of coronary artery disease. Future studies using larger sample sizes, and employing either similar or different analytical strategies may help in elucidating the impact of these polymorphisms on the risk of coronary artery disease.

Conflicts of interest

The authors declare no conflict of interest.

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