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Correlation between polymorphisms in the *IL-17A* and *IL-17F* genes and development of coronary artery disease

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ABSTRACT. A case-control study was conducted to investigate the association between genetic variants of *IL-17A* rs2275913 and *IL-17F* rs763780 and the development of coronary artery disease (CAD) in a Chinese population. A total of 306 individuals with CAD and 306 unaffected individuals were enrolled from the Zhengzhou People's Hospital between May 2012 and May 2014. The *IL-17A* rs2275913 and *IL-17F* rs763780 genes were genotyped by polymerase chain reaction combined with a restriction fragment length polymorphism (PCR-RFLP). Logistic regression analysis revealed that individuals with the AA genotype of rs2275913 were associated with increased risk of CAD, compared to those with the GG genotype in a codominant model [adjusted odds ratio (OR) = 1.96; 95% confidence interval (CI) = 1.10-3.53]. On the other hand, the AA genotype of rs2275913 was correlated with moderately increased risk of CAD compared to the *GG* + *GA* genotype (adjusted OR = 1.76; 95%CI = 1.02-3.07) in a recessive model. However, no significant differences were observed between polymorphisms at the *IL-17F* rs763780 locus and CAD risk, in codominant, dominant, and recessive models. In conclusion, the results of our study suggested that the *IL-17A* rs2275913 polymorphism may affect

the development of CAD; however, no significant association was observed between the *IL-17F* rs763780 polymorphism and risk of CAD.

Key words: *IL-17A*; *IL-17F*; Polymorphism; Coronary artery disease

INTRODUCTION

Cardiovascular disease is one of the major causes of death worldwide, including China; coronary artery disease (CAD) is the most common heart disease causing atherosclerosis (He et al., 2005; WHO, 2015). CAD is caused by complex factors, including multiple genetic and environmental factors and their interactions (Lindahl et al., 2000). The major environmental factors inducing CAD include hypertension, hypercholesterolemia, diabetes, obesity, and smoking and drinking (Go et al., 2014). However, these traditional factors cannot fully predict the incidence of CAD; therefore, genetic factors may contribute to the underlying pathogenesis of CAD (Yang et al., 2014; Yuan et al., 2014; Hou et al., 2015; Xie et al., 2015).

Interleukin-17 (*IL-17*) is a cytokine secreted exclusively by activated T-cells that bridges the adaptive and innate immune systems (Rutitzky et al., 2005; Ishigame et al., 2009). *IL-17A* and *IL-17F* are important members of the *IL-17* cytokine family; these are preferentially produced by helper T 17 (Th17) cells, which are responsible for the pathogenic activity of CD4+ effector cells and multiple proinflammatory mediators (Rutitzky et al., 2005; Ishigame et al., 2009). Previous studies have reported that *IL-17A* could potentially mobilize, recruit, and activate macrophages in atherosclerotic lesions (Eid et al., 2009; Taleb et al., 2009). *IL-17A* rs2275913 and *IL-17F* rs763780 are two common SNP loci in the *IL-17* genes, and these two SNPs could influence the transcriptional regulation of *IL-17*. Previous studies have reported an association between *IL-17A* rs2275913 and *IL-17F* rs763780 and various diseases, such as inflammatory bowel disease, osteoarthritis, tuberculosis, and cancers (Zhang et al., 2013; Han et al., 2014; Bulat-Kardum et al., 2015; Gao et al., 2015). However, only two previous studies have reported an association between the *IL-17A* gene polymorphism and cardiovascular disease (Pei et al., 2009; Zhang et al., 2011). In this study, a case-control study was conducted to investigate the role of the genetic variants of *IL-17A* rs2275913 and *IL-17F* rs763780 in the development of CAD in a Chinese population.

MATERIAL AND METHODS

Study population

A total of 306 individuals with CAD and 306 unaffected individuals were enrolled from the Zhengzhou People's Hospital between May 2012 and May 2014. The CAD cases were diagnosed by angiography between May 2012 and May 2014, and CAD was defined as a diameter stenosis of 50% in any of the main coronary arteries, such as the left main, left anterior descending, left circumflex, or right coronary artery. Subjects with myocardial spasms or a myocardial bridge, congenital heart disease, peripheral artery disease, or those receiving treatment or medication for hypertension or peripheral artery disease, and those diagnosed with any autoimmune-related, renal, or liver diseases, or cancers, were excluded from our study. The mean age at diagnosis of the CAD patients was 61.5 ± 10.5 years.

The controls were randomly selected from among individuals who underwent a regular health examination at our hospital between May 2012 and May 2014. The control subjects had

no diagnostic history of arteriosclerotic lesions and CAD. The mean age of controls at the time of participation in our study was 60.3 ± 9.8 years.

The clinical and demographic information of the CAD patients and control subjects were collected from medical records. A written informed consent was obtained from each subject prior to the study. The collection of blood samples for this study was previously approved by the Ethics Committee of the Henan Provincial People's Hospital.

The demographic characteristics of CAD patients and controls were collected from a self-designed questionnaire through a face-to-face interview; the investigated characteristics included tobacco usage, alcohol consumption, and body mass index (BMI). Clinical data, such as a history of hypertension or diabetes mellitus, total cholesterol (TC) content, low density lipopolysaccharide cholesterol (LDL-c), high density lipopolysaccharide (HDL-c), and triglyceride (TG) content, was collected from the medical records of the subjects.

Genetic analysis

Peripheral venous blood (5 mL) was obtained from each patient and control subject. The collected blood samples were stored at -20°C until analysis. Genomic DNA was extracted from the peripheral blood using the TIANamp Blood DNA kit (Tiangen Biotech Co., Ltd., Beijing, China). *IL-17A* rs2275913 and *IL-17F* rs763780 were genotyped by polymerase chain reaction combined with restriction fragment length polymorphism (PCR-RFLP). The following primers were used for this reaction: *IL-17A* rs2275913, F-5'-GCAGCTCTGCTCAGCTTCTAA-3' and R-5'-TTCAGGGGT GACACCATTTT-3'; and *IL-17F* rs763780, F-5'-CTGTTTCCATCCGTGCAGGTC-3' and R-5'-TGGT GACTGTTGGCTGCACCT-3'. The samples were amplified under the following cycling program: an initial denaturation step at 95°C for 5 min; 30 cycles of annealing with denaturation at 94°C for 30 s, touchdown annealing at 60°C for 30 s, and final annealing at 72°C for 1 min; and a final extension at 72°C for 10 min. The PCR products were verified using a 2% agarose gel stained with ethidium bromide and ultraviolet light.

Statistical analysis

Continuous variables are reported as means ± standard deviation, and categorical variables are shown as frequencies and percentages. The differences between continuous and categorical variables were calculated by two-tailed Student *t*-test and χ^2-test, respectively. Deviations from the Hardy-Weinberg equilibrium (HWE) of genetic distributions of *IL-17A* rs2275913 and *IL-17F* rs763780 in the controls were evaluated by the χ^2-test. Multivariate conditional logistic regression analysis was performed to analyze the association between the *IL-17A* rs2275913 and *IL-17F* rs763780 polymorphisms and risk of CAD; the results were expressed as odds ratio (OR) and 95% confidence interval (CI) after adjusting for potential confounding factors (age, hypertension, diabetes mellitus, alcohol consumption, tobacco usage, and body mass index). Homozygotes of the most frequent genotype of *IL-17A* rs2275913 and *IL-17F* rs763780 were used as the reference group. Codominant and recessive models were used to assess the association between *IL-17A* rs2275913 and *IL-17F* rs763780 polymorphisms and risk of CAD. All P values were two sided, and P values <0.05 were considered to indicate statistically significant differences. All statistical analyses in this study were performed using the SPSS software (v.16.0; SPSS, Chicago, IL, USA) for Windows.

RESULTS

The demographic and clinical characteristics of CAD patients and control subjects have been summarized in Table 1. As expected, we observed no significant differences between the CAD patients and control subjects with respect to the gender, age, and alcohol consumption rate (P > 0.05). The CAD patients were more likely to have hypertension, diabetes mellitus, a higher BMI, and a habit of tobacco usage (P < 0.05) compared to control subjects. Moreover, CAD patients showed higher levels of TC and TG compared to control subjects (P < 0.05); however, CAD patients showed lower levels of LDL-c and HDL-c.

Table 1. Demographic and clinical characteristics of the included cases and control subjects.

	CAD cases (N = 306)	%	Controls (N = 306)	%	χ^2 test or *t* test	P value
Mean age, years	61.5 ± 10.5			60.3 ± 9.8	1.46	0.07
Gender						
Male	234	76.47	234	76.47		
Female	72	23.53	72	23.53	0.00	1.00
Hypertension						
No	127	41.50	210	68.63		
Yes	179	58.50	96	31.37	45.49	<0.001
Diabetes mellitus						
No	192	62.75	271	88.56		
Yes	114	37.25	35	11.44	55.37	<0.001
Alcohol drinking						
Never	148	48.37	170	55.56		
Current or ever	158	51.63	136	44.44	3.17	0.08
Tobacco smoking						
Never	130	42.48	222	72.55		
Current or ever	176	57.52	84	27.45	56.6	<0.001
Body mass index (kg/m^2)	26.23 ± 3.52		23.85 ± 3.21		8.74	<0.001
TC (mM)	4.72 ± 1.13		4.51 ± 0.94		2.5	0.006
LDL-c (mM)	2.16 ± 1.12		3.14 ± 1.06		12.99	<0.001
HDL-c (mM)	1.15 ± 0.55		1.63 ± 0.47		11.61	<0.001
TG (mM)	1.92 ± 0.95		1.45 ± 1.04		5.84	<0.001

CAD, coronary artery disease; TC, total cholesterol; LDL-c, low-density lipopolysaccharide cholesterol; HDL-c, high-density lipopolysaccharide cholesterol; TG, triglyceride.

The genotype distributions of *IL-17A* rs2275913 were found to be in line with the HWE in the control group, while those of *IL-17F* rs763780 were not (Table 2). The minor allele frequencies (MAF) of the six SNPs in the control samples were similar to those uploaded to the database (http://www.ncbi.nlm.nih.gov/snp). The *GG*, *GA*, and *AA* genotypes of the *IL-17A* rs2275913 locus were exhibited by 123 (40.20%), 140 (45.75%), and 43 (14.05%) of the CAD patients, and 146 (47.71%), 134 (43.79%), and 26 (8.50%) of the control subjects, respectively. The *TT*, *TC*, and *CC* genotypes of the *IL-17F* rs763780 locus were shown by 241 (78.76%), 45 (14.71%), and 20 (6.54%) CAD patients and 252 (82.35%), 41 (13.40%), and 13 (4.25%) control subjects, respectively. We observed significant differences in the genotype frequencies of *IL-17A* rs2275913 between CAD patients and controls (χ^2 = 6.29, P value = 0.04).

The logistic regression analysis revealed that individuals with the *AA* genotype of rs2275913 were associated with increased risk of CAD compared to the *GG* genotype in a co-dominant model; in such a case, the adjusted OR (95%CI) was 1.96 (1.10-3.53) (Table 3). On the other hand, the *AA* genotype of rs2275913 was correlated with moderately increased risk of CAD compared to the *GG* + *GA* genotype in a recessive model (adjusted OR = 1.76; 95%CI = 1.02-

3.07). However, we observed no significant differences in polymorphisms at the *IL-17F* rs763780 locus and CAD risk between codominant, dominant, and recessive models.

We also performed a gene-environmental association of *IL-17A* rs2275913 polymorphism with age, hypertension, diabetes mellitus, alcohol consumption, tobacco usage, and BMI and the risk of CAD; however, we did not find a significant association between the *IL-17A* rs2275913 polymorphism and these lifestyle characteristics (all P values > 0.05).

Table 2. *IL-17A* rs2275913 and *IL-17F* rs763780 genotype distribution in coronary artery disease (CAD) cases and controls.

IL-17 gene	Base change	Patients	%	Controls	%	χ^2	P value	P value for HWE	MAF	
									In database	In controls
rs2275913										
GG		123	40.20	146	47.71					
GA		140	45.75	134	43.79					
AA	G>A	43	14.05	26	8.50	6.29	0.04	0.54	0.2927	0.3039
rs763780										
TT		241	78.76	252	82.35					
TC		45	14.70	41	13.40					
CC	T>C	20	6.54	13	4.25	1.91	0.38	<0.001	0.0935	0.1095

HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency.

Table 3. Association between *IL-17A* rs2275913 and *IL-17F* rs763780 gene polymorphisms and risk of coronary artery disease.

	IL-17	Cases	%	Controls	%	OR (95%CI)[1]	P value
Codominant model	rs2275913						
	GG	123	40.2	146	47.71	Ref.	-
	GA	140	45.75	134	43.79	1.24 (0.87-1.76)	0.21
	AA	43	14.05	26	8.5	1.96 (1.10-3.53)	0.01
Dominant model	GG	123	40.2	146	47.71	Ref.	-
	GA + AA	183	59.8	160	52.29	1.36 (0.97-1.89)	0.06
Recessive model	GG + GA	263	85.95	280	91.5	Ref.	-
	AA	43	14.05	26	8.5	1.76 (1.02-3.07)	0.03
	rs763780						
Codominant model	TT	241	78.76	252	82.35	Ref.	-
	TC	45	14.71	41	13.4	1.15 (0.71-1.87)	0.56
	CC	20	6.54	13	4.25	1.61 (0.74-3.60)	0.19
Dominant model	TT	241	78.76	252	82.35	Ref.	-
	TC + CC	65	21.25	54	17.65	1.26 (0.83-1.92)	0.26
Recessive model							
	TT + TC	286	93.47	293	95.75	Ref.	-
	CC	20	6.54	13	4.25	1.58 (0.73-3.52)	0.21

[1]Adjusted for gender, age, hypertension, diabetes mellitus, tobacco smoking and body mass index levels; OD, odds ratio; CI, confidence interval.

DISCUSSION

Cytokines are involved in modifying the immune response, and play a role in balancing the pro-inflammatory and anti-inflammatory stimuli during cardiovascular disease. Two previous studies have reported the association between polymorphisms in the *IL-17* gene and cardiovascular disease (Pei et al., 2009; Zhang et al., 2011). Therefore, the *IL-17* gene polymorphisms could influence the susceptibility to CAD inflammation through the level of pro-inflammatory and neutrophil-mobilizing

cytokines in the plasma. The role of *IL-17* gene polymorphisms in the development of CAD was assessed in a Chinese population.

In our study, the AA genotype and A allele of the *IL-17A* rs2275913 polymorphisms were found to be associated with an increased risk of CAD in the codominant and recessive models of multivariate analysis, even after adjusting for the conventional risk factors of CAD. Previous experimental studies have reported that the expression of *IL-17A* could induce pro-inflammatory cytokine and chemokine expression, resulting in neutrophil infiltration-related inflammation and the development of atherosclerosis (Eid et al., 2009; Taleb et al., 2009; de Boer et al., 2010), which supports the biological mechanisms of *IL-17A* in the development of atherosclerosis and CAD.

Zhang et al. (2011) assessed the possible involvement of polymorphisms in the *IL-17A* rs4711998, rs3819024, rs2275913, rs8193037, and rs3819025 loci in the induction of CAD in order to determine an association between polymorphisms in the *IL-17* gene, and risk of atherosclerosis; they reported that *IL-17A* rs8193037 is associated with increased risk of CAD in a Han Chinese population (Zhang et al., 2011). However, Pei et al. (2009) reported that the *IL-17F* His161Arg rs12046844 polymorphism is not associated with a pathogenesis of myocardial infarction. In this study, we investigated the association between two common SNPs (rs2275913 and rs763780) in the *IL-17* gene and CAD; the results of this study suggested that the *IL-17A* rs2275913 polymorphism was correlated with the development of CAD in a Chinese population. Most importantly, the rs2275913 polymorphism is located at the 5′-region of the *IL-17A* gene, and may therefore regulate gene transcription (Qinghai et al., 2014). Hence, the functions of this SNP could influence the expression of *IL-17*. Therefore, further larger sample studies must be conducted to confirm our results.

Several limitations should be considered in our study. Firstly, the cases and controls were selected from a single hospital; in addition, the *IL-17F* rs763780 was in line with the HWE in the control group, which suggested that the selected controls may not be representative of the general population. Secondly, genetic polymorphisms in addition to the *IL-17* gene polymorphisms may influence the development of CAD. Thirdly, the sample size of this study was relatively small, which may limit the statistical power of determining the differences between the groups. In addition, only two previous studies have reported an association between *IL-17A* gene polymorphisms and risk of CAD; therefore, there is very limited evidence to confirm the results of our study. Therefore, larger sample size studies are required to confirm the results of our study.

In conclusion, the results of our study suggest that the *IL-17A* rs2275913 polymorphism may affect the development of CAD in codominant and recessive models. However, no significant association was found between the *IL-17F* rs763780 polymorphism and risk of CAD. Further studies with larger sample sizes are therefore required to confirm our results.

Conflicts of interest

The authors declare no conflict of interest.

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