Int J Clin Exp Pathol 2016;9(5):5673-5679
www.ijcep.com /ISSN:1936-2625/IJCEP0019435

Original Article

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

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Received November 9, 2015; Accepted March 1, 2016; Epub May 1, 2016; Published May 15, 2016

Abstract: We conducted a case-control study to investigate the association between *IL-10* gene polymorphisms and development of CAD in a Chinese population. Between May 2012 and June 2014, a total of 220 patients suffered with CAD and 236 control subjects were selected from the Department of Cardiology of Huaihe Hospital of Henan University. The genotyping of IL-10-1082A/G and -592A/C were analyzed using polymerase chain reaction coupled with restriction fragment length polymorphism (PCR-RFLP). By unconditional multiple logistic regression analysis, we found that the CC genotype of *IL-10*-1082A/G was correlated with the AA genotype (OR=2.55, 95% CI=1.33-4.96). In dominant model, the AC+CC genotype of *IL-10*-1082A/G was associated with development of CAD (OR=1.55, 95% CI=1.05-2.29). In recessive model, the CC genotype of *IL-10*-1082A/G was correlated with the risk of CAD (OR=2.19, 95% CI=1.19-4.13). However, we did not find significant association between *IL-10*-592A/C polymorphisms and risk of CAD. In conclusion, our study suggests that the *IL-10*-1082A/G polymorphism is associated with CAD risk in codominant, dominant and recessive models.

Keywords: *IL-10*, -1082A/G, -592A/C, polymorphism, CAD

Introduction

Coronary artery disease is one of the most serious diseases, which is related with high mortality and morbidity worldwide, and also in China [1, 2]. As we know, the CAD is caused by multiple lifestyle and environmental factors and their interactions, such as high sugar dietary, tobacco smoking, high cholesterol, high blood pressure, diabetes, obesity and low high-density lipoprotein cholesterol as well as coagulant function abnormality [3]. However, not all individuals with similar risk factors would suffer from CAD, which suggested that genetic factors may contribute to the susceptibility to CAD. Previous large-scale genome-wide associated studies have shown that about 50 risk loci for the development of CAD [4-7].

Inflammation has been reported to be associated with the pathogenesis of CAD [8, 9]. Previous molecular studies have reported that many interleukin factors are involved in the development of CAD, such as interleukin-6 (IL-6), IL-1β, IL-8 and IL-17A genes [10, 11]. *IL-10* is one member of immunoregulatory cytokine, and this gene is produced by Th2 cells and regulatory T cells as well as and monocytes/macrophages. The *IL-10* gene is mapped to human chromosome 1 (1q31-1q32). Previous studies have reported that the *IL-10* gene polymorphisms are associated with development of cardiovascular disease, but the results are inconclusive [11-17]. In our study, we conducted a case-control study to investigate the association between *IL-10* gene polymorphisms and development of CAD in a Chinese population.

Patients and methods

Study subjects

Between May 2012 and June 2014, a total of 220 patients suffered with CAD were selected from the Department of Cardiology of Huaihe Hospital of Henan University. All the CAD patients were confirmed by coronary angiography. The CAD is defined as those cases with

Table 1. Primers, restriction enzymes and digested fragments for IL-10-1082A/G and -592A/C polymorphisms

Gene polymorphism	Primers	Length of digested fragment	Restriction enzyme	Digested fragment
-1082A/G	5'-CCAAGACAACACTACTAAGGCTCCTTT-3' 5'-GCTTCTTATATGCTAGTCAGGTA-3'	139 bp	*Mn*II	G allele: 106 bp and 33 bp; A allele: 139 bp
-592A/C	5'-GGTGAGCACTACCTGACTAGC3-3' 5'-CCTAGGTCACAGTGACGTGG-3'	412 bp	RsaI	A allele: 176 bp and 236 bp; C allele: 412 bp

above 70% luminal stenosis in one of the main vessels, having the typical angina symptoms without any aortic valvular diseases, and having a history of myocardial infarction. Patients who had experimental myocardial spasms, myocardial bridge, congenital heart disease and childhood hypertension as well as type one diabetes mellitus were excluded from our study.

236 control subjects were randomly selected from individuals who underwent physical examinations in the Department of Cardiology of Huaihe Hospital of Henan University between May 2012 and June 2014. Controls were confirmed to be free of atherosclerotic lesions and CAD by angiography.

The demographic and clinical characteristics of patients with CAD and control subjects were collected from medical records, including sex, age, tobacco smoking, alcohol drinking, BMI, hypertension, type 2 diabetes mellitus, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) as well as triglyceride (TG). The study was performed with the permission of the Institutional Review Board of the Huaihe Hospital of Henan University. Written informed consents were obtained from all the CAD patients and control subjects. Ethical approval for this study conformed to the standards of the Declaration of Helsinki.

Genetic analysis

Each subject was asked to provide 5 ml peripheral blood, and the blood samples were collected on EDTA and extracted using the TIANamp Blood DNA Kit (Tiangen, Beijing, China) following the manufacturer's instructions. The extracted DNA was stored at -20°C until further use. The genotyping of *IL-10*-1082A/G and -592A/C were analyzed using polymerase chain reaction coupled with restriction fragment length polymorphism (PCR-RFLP). The primers, length of digested fragment and restriction enzymes of *IL-10*-1082A/G and -592A/C were shown in **Table 1**. The PCR cycles were using the following program: one cycle of 95°C with 5 minutes for denaturation, 35 cycles at 94°C with 40 seconds for denaturation, 60 seconds at 55°C for annealing, and 40 seconds at 72°C for extension, and 10 minutes at 72°C for a final extension. The amplified products were confirmed using 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

Statistical analysis

Differences between demographic and clinical characteristics of patients with CAD and control subjects were analyzed using chi-square test or student t test. The goodness-of-fit χ^2-test was taken to assess whether the genotype distributions in the patients and controls were deviation from the Hardy-Weinberg equilibrium (HWE). Multiple logistic regression analysis was taken to assess the association between *IL-10*-1082A/G and -592A/C gene polymorphisms and development of CAD. Multiple logistic regression analysis was performed to analyze the correlation between *IL-10*-1082A/G and -592A/C gene polymorphisms and susceptibility to CAD, and the results were analyzed using odds ratio (OR) and their related 95% confidence intervals (CIs). All the analysis was conducted with SPSS 16.0 (SPSS Inc., Chicago, IL, USA). A P values <0.05 was considered as a statistically significance.

Results

The lifestyle and clinical characteristics of patients with CAD and control subjects were shown in **Table 2**. The mean age of patients with CAD and controls were 62.53±10.46 and 58.39±9.60 years, respectively. There were 140 (63.8%) females and 80 (36.2%) males in CAD patients, and 131 (55.8%) females and 105 (44.2%) in controls. By chi-square test or t test, we found that patients with CAD were

Table 2. Characteristics of CAD patients and control subjects

Characteristics	CAD patients	%	Controls	%	χ^2-test or t test	*P* value
Age, years						
<60	104	47.3	124	52.6		
≥60	116	52.7	112	47.4	1.26	0.26
Gender						
Female	140	63.8	131	55.8		
Male	80	36.2	105	44.2	3.12	0.08
Tobacco smoking						
No	98	44.5	160	67.7		
Yes	122	55.5	76	32.3	25.06	<0.001
BMI, kg/m^2						
<24	101	46.1	157	66.5		
≥24	119	53.9	79	33.5	19.70	<0.001
Hypertension						
No	93	42.2	171	72.3		
Yes	127	57.8	65	27.7	42.56	<0.001
Type 2 diabetes						
No	150	68.3	207	87.5		
Yes	70	31.7	29	12.5	25.55	<0.001
TC, mg/dL		196.55±36.60		172.22±34.63	7.29	<0.001
LDL-c, mg/dL		112.82±24.74		102.70±23.66	4.46	<0.001
HDL-c, mg/dL		38.51±18.30		42.89±12.62	2.99	0.002
TG, mg/dL		137.73±40.26		115.62±25.73	7.04	<0.001

more likely to be tobacco smokers (χ^2= 25.06, *P*<0.001), have higher BMI (χ^2=19.70, *P*<0.001), and suffer from hypertension (χ^2= 42.56, *P*<0.001) and type 2 diabetes (χ^2= 25.55, *P*<0.001). Moreover, patients with CAD had higher TC (*t*=7.29, *P*<0.001), LDL-c (*t*= 4.46, *P*<0.001) and TG (*t*=2.99, *P*<0.001), and had lower HDL-c (*t*=7.04, *P*<0.001).

Genotype distributions of *IL-10*-1082A/G and -592A/C gene polymorphisms were shown in **Table 3**. The genotype distributions of *IL-10*-1082A/G and -592A/C in the CAD patients confirmed with the Hardy-Weinberg equilibrium (*P* values for HWE were 0.34 and 0.83, respectively), and they were also in line with Hardy-Weinberg equilibrium in controls (*P* values for HWE were 0.91 and 0.88, respectively). By chi-square test, a significant different was found in the distributions of *IL-10*-1082A/G genotypes (χ^2=9.54, *P*=0.008). By unconditional multiple logistic regression analysis, we found that the CC genotype of *IL-10*-1082A/G was correlated with the development of CAD, when compared with the AA genotype (OR=2.55, 95% CI=1.33-4.96). In dominant model, the AC+CC genotype of *IL-10*-1082A/G was associated with development of CAD (OR=1.55, 95% CI=1.05-2.29). In recessive model, the CC genotype of *IL-10*-1082A/G was correlated with the risk of CAD (OR=2.19, 95% CI=1.19-4.13). However, we did not find significant association between *IL-10*-592A/C polymorphisms and risk of CAD.

Discussion

It is well known that *IL-10* is an important mediator of *in vivo* inflammatory reactions, and this gene is associated with the inflammatory response to CAD, and is associated with cardiovascular disease [18, 19]. Moreover, a previous study reported that the serum levels of *IL-10* significantly influence the pathophysiological processes in atherosclerosis and heart failure [20, 21]. Single nucleotide polymorphism (SNP) means the gene sequence of a single nucleotide bases insert, missing or replacement the nucleic acid sequence polymorphism. About millions of SNP, more than 1% of the population shows gene polymorphisms, including the conversion of single-base transversion in a single base insertion/deletion, and other forms of

Table 3. Genotype distributions of *IL-10*-1082A/G and -592A/C gene polymorphisms between patients with CAD and control subjects

IL-10	Patients N=220	%	Controls N=236	%	P for HWE		χ^2 test	*P* value	OR (95% CI)[1]	*P* value
					In cases	In controls				
-1082A/G										
Co-dominant										
AA	85	38.64	117	49.58					1.0 (Reference)	
AC	98	44.55	100	42.37					1.35 (0.89-2.04)	0.14
CC	37	16.82	20	8.05	0.34	0.83	9.54	0.008	2.55 (1.33-4.96)	0.002
Dominant										
AA	85	38.64	117	49.58					1.0 (Reference)	
AC+CC	135	61.36	120	50.42			5.33	0.02	1.55 (1.05-2.29)	0.02
Recessive										
AA+AC	183	83.18	217	91.95					1.0 (Reference)	
CC	37	16.82	20	8.05			7.34	0.01	2.19 (1.19-4.13)	0.01
-592A/C										
Co-dominant										
AA	80	36.4	98	41.56					1.0 (Reference)	
AG	106	48.2	109	46.32					1.19 (0.78-1.81)	0.39
GG	34	15.4	29	12.12	0.91	0.88	1.7	0.43	1.44 (0.77-2.67)	0.22
Dominant										
AA	80	36.4	98	41.56					1.0 (Reference)	
AG+GG	140	63.6	138	58.44			1.27	0.26	1.24 (0.84-1.85)	0.26
Recessive										
AA+AG	186	84.6	207	87.88					1.0 (Reference)	
GG	34	15.4	29	12.12			0.96	0.33	1.30 (0.74-2.31)	0.33

[1]Adjusted for sex, age, tobacco smoking, BMI, hypertension, type 2 diabetes, TC, LDL-c, HDL-c and TG. HWE: Hardy-Weinberg equilibrium. OR: odds ratio; CI: confidence Interval.

performance [22]. The genetic polymorphism can change the structure of the gene expression product and quantity, and ultimately affect the function of this gene. In our study, we conducted a study to investigate the association between IL-*10*-1082A/G and -592A/C polymorphisms and development of CAD, and we found that the CC genotype of *IL-10*-1082A/G was associated with CAD risk, and *IL-10*-1082A/G polymorphism was correlated with this disease in dominant and recessive models.

The IL-10 gene is located on chromosome 1 (q31-32), and this gene is reported to be a multifunction cytokine and is produced by immune and many nonimmune cells [23]. Previous studies have reported the association between *IL-10* gene polymorphisms and the development of various cardiovascular diseases [13, 16, 23-28]. Rehman et al. conducted a case-control study in a Pakistani population, and they found that *IL-10*-1082A/G gene polymorphism was associated with the development of reheumatic heart disease [24]. Lin et al. investigate the role of *IL-10*-1082A/G genetic polymorphisms in the development of coronary artery aneurysms, and they found that *IL-10*-1082A/G gene polymorphisms may be related to the development of this disease [16]. He et al. conducted a study in a Chinese population, and they found that the IL-*10*-1082A/G and -592A/C gene polymorphisms could not influence the development of left main coronary artery disease (He et al., 2014). Qi et al. suggests that IL-10 gene polymorphism was associated with the susceptibility of cerebral thrombosis in a Chinese population [26]. Elsaid et al. conducted a study in Egyptian patients with CAD and 143 unrelated healthy subjects, and they *IL-10* gene polymorphisms was associated with an increased prevalence of CAD [27]. Yu et al. reported that the promoter region polymorphisms of *IL-10* gene are associated with the risk of atherosclerosis [28]. A recent meta-anal-

ysis conducted by Jin et al. and included seven studies, and this meta-analysis found that IL-*10*-1082A/G gene polymorphism is associated with susceptibility to CAD in Asians [13]. In our study we found that the *IL-10*-1082A/G polymorphism is associated with the development of CAD. Such discrepancies between the results of previous studies may have been caused by differences in populations, study designs, and sample sizes.

Two limitations should be considered in our study. First, patients and controls were selected from a single hospital, which may cause selection bias in this study. However, this bias could be corrected, because the genotype distributions of *IL-10*-1082A/G and -592A/C in the CAD patients confirmed with the Hardy-Weinberg equilibrium in the controls. Second, the sample size relative small, which might suffer from lack of power to find association of *IL-10* gene polymorphisms with the risk of CAD.

In conclusion, our study suggests that the *IL-10*-1082A/G polymorphism is associated with CAD risk in codominant, dominant and recessive models. Further studies with large samples are greatly needed to verify our results.

Acknowledgements

We thank for the funding from Science and technology research key project of education department of Henan Province: Application of fractional flow reserve (FFR) in multivessel coronary artery disease (13A320055).

Disclosure of conflict of interest

None.

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