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Association between IL-4 genetic polymorphism and risk of coronary artery disease in a Chinese population.

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Abstract

We aimed to investigate the association between IL-4 genetic polymorphisms (IL-4 rs2243250, rs2227284, rs2243267, rs2243270) and risk of CAD in a Han Chinese individual. A total of 366 patients with coronary artery disease patients and 366 controls were collected from July 2014 to July 2016. SNP genotyping of IL-4 rs2243250, rs2227284, rs2243267 and rs2243270 was done in a 384-well plate format on the sequenom Mass ARRAY platform. Conditional logistic regression analysis was performed to analyze the relationship between the four SNPs and risk of CAD. We found that those with BMI ≥ 24 (adjusted OR=2.71, 95% CI=1.70-4.30), T2DM (adjusted OR=2.59, 95% CI=1.61-4.17) or hypertension (adjusted OR=2.10, 95% CI=1.47-3.00) were associated with an increased risk of CAD compared with the reference group. The TT (adjusted OR=2.86, 95% CI=1.75-4.68) and CT+TT (adjusted OR=1.39, 95% CI=1.02-1.90) genotypes of rs2243250 were correlated with an elevated risk of CAD when compared with the CC genotype. The GG genotype of rs2243270 was associated with a higher risk of CAD in comparison to the AA genotype (adjusted OR=1.97, 95% CI=1.22-3.18). Our study suggests that IL-4 may be a useful biomarker for prediction of the susceptibility of this study.

Keywords: IL-4, Polymorphism, Coronary artery disease (CAD).

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Introduction

Coronary Artery Disease (CAD) is the main cause of death worldwide and the prevalence is increasing in China [1,2]. Coronary artery disease displays a wide range of clinical manifestations, such as subclinical asymptomatic atherosclerosis and some clinical complications [3-5]. Currently, CAD is regarded as one of the most leading cause of death and disability worldwide [6]. The precision in estimating the risk for CAD is crucial for future therapy decision and prevention strategies. Improving the accuracy of risk prediction is particularly important in patients with CAD risk [7]. The prevalence of CAD is usually high in individuals with many lifestyle and dietary factors [8]. Therefore, the use of emergent biomarkers could contribute to identify individuals at high-risk, and pose a critical challenge for CAD prevention.

There is extensive evidence supporting a role of inflammatory response in the pathogenesis of CAD and in the natural history of atherosclerosis [9,10]. Cardiovascular events are more common in patients with high circulating levels of several inflammatory markers, such as hs-C reactive protein (hs-CRP) [11]. The Interleukins (IL) mediate various effects in inflammation. Interleukin 4 (IL-4) is mainly secreted by activated T cells and mononuclear cells [12]. In humans, diminished products of IL-4 are considered to contribute to the autoimmune diseases [13]. As well, polymorphisms of IL-4 gene coding for the cytokine IL-4 have been thought to influence the susceptibility or protection in autoimmune diseases [14-16]. However, few studies reported an association of IL-4 expression and polymorphisms with risk of CAD [17,18]. In the present study, we conducted a case-control study to investigate the association between IL-4 genetic polymorphisms (IL-4 rs2243250, rs2227284, rs2243267 and rs2243270) and risk of CAD in Han Chinese individuals.

Methods

Study subjects

A total of 366 patients with coronary artery disease patients were collected from July 2014 to July 2016 in the department of cardiology of Affiliated Hospital of Hebei University of Engineering. Coronary angiography was performed and all the patients were confirmed to be CAD according to the WHO criteria for diagnosis of patients with CAD in 1979.

Simultaneously, a total of 366 control group were selected from the outpatient clinics and health examination center of Affiliated Hospital of Hebei University of Engineering, and

they matched with patients with age (± 5 y). All the controls received health examination, and they were confirmed to be free of CAD. All respondents voluntary participated in our study and signed informed consent forms before enrolment. This study was approved by the ethics committee of the Affiliated Hospital of Hebei University of Engineering.

All the subjects were unrelated Chinese Han population; and we excluded those with systemic inflammation, rheumatic disease, serious heart failure, malignant tumors, thyroid function derangement and autoimmune diseases, but did not exclude hypertension and diabetes.

Demographic and lifestyle characteristics were collected from the questionnaire survey, including weight, height, alcohol drinking habit and tobacco smoking habit. The clinical information collected from medical records, such as family history of cardiovascular disease, hypertension, Type 2 Diabetes Mellitus (T2DM), Total Cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein cholesterol (LDL-c) and High Density Lipoprotein cholesterol (HDL-c). Tobacco smoking status was divided into never smoking and ever smoking. Ever smoking was defined as those who smoked more than 20 cigarettes per week and continued for half a year, and the remaining respondents were considered as never smoking. The alcohol drinking status was divided into never drinking and ever drinking. Ever drinking was defined as those who drank more than 50 g white wine or half a bottle of beer and lasted for half a year. T2DM was defined as those with a fast plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or with a glucose tolerance test (two hours after the oral dose plasma glucose) ≥ 11.1 mol/l (200 mg/dl). Hypertension was defined as those with systolic pressure ≥140 mmHg or diastolic pressure ≥ 90 mmHg.

Three ml of peripheral venous blood was collected from each subject. The blood samples were centrifuged with 1500r/min to separate serum, and stored at -80°C for lipid analysis; the white cell was handled with Blood DNA extraction kit to extract genomic DNA, and then the DNA samples were stored at -20°C preservation for genotype analysis.

SNP genotyping of IL-4 rs2243250, rs2227284, rs2243267 and rs2243270 was done in a 384-well plate format on the sequenom MassARRAY platform (Sequenom, San Diego, USA). Primers for polymerase chain reaction amplification and single base extension assays of the four SNPs were designed by Sequenom Assay Design 3.1 software. The genomic DNA of IL-4 rs2243250, rs2227284, rs2243267 and rs2243270 was amplified with an initial denaturation at 95°C for 2 min; 45 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 60 s; a final extension at 72°C for 5 min. Then the SAP and iPLEX reactions were then carried out. Then the PCR products were desalted, and finally the products were dispensed to a SpectroCHIP and analyzed with MALDI-TOF MS.

Statistical analysis

Data analysis was performed with the software IBM SPSS Statistics for Windows, Version 20.0. (IBM Corp. Armonk, NY, USA). Two tailed $P<0.05$ was considered to be statistical significant difference. Continuous variables are shown by means ± standard deviations (SD) and categorical variables are displayed by percentages and frequencies (%). First, comparison of demographic, lifestyle and clinical characteristics between the two groups were analyzed by Chi-square test or student t- test. Second, the genotype frequencies of IL-4 rs2243250, rs2227284, rs2243267 and rs2243270 between the two study groups were compared by Chi-square test and the Hardy-Weinberg equilibrium of the four SNPs was assessed with Chi-square (χ^2) test with one degree of freedom. Relationship of the four SNPs with risk of CAD was estimated by conditional multivariate logistic regression analysis, and the results were displayed by Odds Ratios (ORs) and 95% Confidence Intervals (CIs). The OR was adjusted for potential confounding factors, such as sex, age, BMI and history of T2DM and hypertension.

Results

Comparison with controls, patients with CAD were more likely to have higher BMI (χ^2=43.84, $P<0.001$), have a history of T2DM (χ^2=22.98, $P<0.001$) and hypertension (χ^2=23.18, $P<0.001$) (Table 1). Moreover, patients with CAD had a higher level of TC (t=2.76, P=0.01), TG (t=3.99, $P<0.001$) and LDL-c (t=5.80, $P<0.001$) and had a lower level of HDL-c (t=-6.83, $P<0.001$).

Table 1. *Comparison of the social demographic, lifestyle and clinical characteristics between the two study groups.*

Variables	CAD patients N=366	%	Controls N=366	%	t-test or χ^2	P value
Age, years		63.38 ± 9.68		63.34 ± 9.20	0.05	0.96
<60	144	39.34	121	33.06		
≥ 60	222	60.66	245	66.94	3.13	0.08
Gender						
Female	146	39.89	161	43.99		
Male	220	60.11	205	56.01	1.26	0.26

BMI, kg/m^2		23.84 ± 3.13		22.52 ± 2.63	6.19	<0.001
<24	183	50	270	73.77		
≥ 24	183	50	96	26.23	43.84	<0.001
T2DM						
No	289	78.96	335	91.53		
Yes	77	21.04	31	8.47	22.98	<0.001
Hypertension						
No	231	63.11	290	79.23		
Yes	135	36.89	76	20.77	23.18	<0.001
Tobacco smoking						
Never	174	47.54	198	54.1		
Ever	192	52.46	168	45.9	3.15	0.08
Alcohol drinking						
Never	163	44.54	189	51.64		
Ever	203	55.46	177	48.36	3.7	0.06
Family history of cardiovascular disease						
No	349	95.36	358	97.81		
Yes	17	4.64	8	2.19	3.36	0.07
TC (mg/dL)		193.29 ± 37.11		185.63 ± 37.87	2.76	0.01
TG (mg/dL)		128.32 ± 33.98		118.87 ± 29.95	3.99	<0.001
LDL-c (mg/dL)		116.19 ± 30.87		102.56 ± 32.73	5.8	<0.001
HDL-c (mg/dL)		39.57 ± 7.31		43.53 ± 8.34	-6.83	<0.001

We observed that the genotype frequencies of IL-4 rs2243250 (χ^2=19.78, P<0.001) and rs2243270 (χ^2=8.53, P=0.01) showed significant differences between patients and controls. However, the genotype distributions of the other four SNPs did not show significant differences in the study groups. All the four SNPs of IL-4 were not deviated from the Hardy-Weinberg equilibrium in controls (P>0.05) (Table 2).

Table 2. *Genotype frequencies of IL-4 rs2243250, rs2227284, rs2243267 and rs2243270 between the two study groups.*

Gene polymorphisms	CAD patients	%	Controls	%	χ^2 value	P value	HWE in controls
rs2243250							
CC	158	43.17	126	34.43	19.78	<0.001	0.35
CT	171	46.72	160	43.72			
TT	37	10.11	80	21.86			
rs2227284							
TT	116	31.69	129	35.25	1.15	0.56	0.56
TG	178	48.63	172	46.99			
GG	72	19.67	65	17.76			
rs2243267							
GG	148	40.44	145	39.62	0.05	0.98	0.16

GC	150	40.98	152	41.53			
CC	68	18.58	69	18.85			
rs2243270							
AA	149	40.71	178	48.63	8.53	0.01	0.08
AG	147	40.16	144	39.34			
GG	70	19.13	44	12.02			

By conditional logistic regression analysis, we found that those with BMI ≥ 24 (adjusted OR=2.71, 95% CI=1.70-4.30), T2DM (adjusted OR=2.59, 95% CI=1.61-4.17) or hypertension (adjusted OR=2.10, 95% CI=1.47-3.00) were associated with an increased risk of CAD compared with the reference group (Table 3). Moreover, the TT (adjusted OR=2.86, 95% CI=1.75-4.68) and CT+TT (adjusted OR=1.39, 95% CI=1.02-1.90) genotypes of rs2243250 were correlated with an elevated risk of CAD when compared with the CC genotype. The GG genotype of rs2243270 was associated with a higher risk of CAD in comparison to the AA genotype (adjusted OR=1.97, 95% CI=1.22-3.18).

Table 3. *Logistic regression analysis for the risk factors of CAD.*

Variables	β	S.E.	Wals	Adjusted OR (95% CI)	P value
BMI ≥ 24, kg/m^2	0.99	0.24	17.64	2.71 (1.70-4.30)	<0.001
T2DM	0.95	0.24	15.26	2.59 (1.61-4.17)	<0.001
Hypertension	0.74	0.18	16.4	2.10 (1.47-3.00)	<0.001
rs2243250					
CC				1.0	-
CT	0.09	0.18	0.28	1.10 (0.77-1.56)	0.6
TT	1.05	0.25	17.42	2.86 (1.75-4.68)	<0.001
CT+TT	0.33	0.16	4.37	1.39 (1.02-1.90)	0.037
rs2227284					
TT				1.0	-
TG	0.11	0.18	0.33	1.11 (0.78-1.59)	0.57
GG	0.09	0.24	0.14	1.09 (0.69-1.74)	0.71
TG+GG	0.12	0.16	0.56	1.13 (0.82-1.56)	0.46
rs2243267					
GG				1.0	-
GC	-0.08	0.18	0.18	0.93 (0.65-1.32)	0.67
CC	-0.13	0.23	0.35	0.87 (0.56-1.37)	0.56
GC+CC	-0.05	0.16	0.1	0.95 (0.70-1.30)	0.75
rs2243270					
AA				1.0	-
AG	0.17	0.18	0.97	1.19 (0.84-1.68)	0.32
GG	0.68	0.24	7.73	1.97 (1.22-3.18)	0.005
AG+GG	0.24	0.15	2.39	1.27 (0.94-1.72)	0.12

Discussion

Genetic polymorphisms may affect the expression and activity of IL-4 and influence the development and progression of disease. In this study, we found that IL-4 rs2243250 and rs2243270 were associated with risk of CAD.

It is reported that inflammation is of major importance for the development of coronary artery disease [19]. Inflammatory cells and signaling molecules play an important role in the pathogenesis of this disease through modulating the arterial wall, promoting lipoprotein retention and plaque formation [19]. Previous studies have indicated that several inflammatory biomarkers could forecast cardiovascular outcome [20,21]. In our study, we found that rs2243250 and rs2243270 were positively associated with the risk of CAD. With regard to rs2243250 being located in the translation start site, we found that individuals with rare TT genotype and CT genotype showed a higher tendency to develop CAD than those with CC homozygous. The variant allele of rs2243250 has been identified as a functional polymorphism being relevant with increased IL-4 transcriptional and translational activity. Rosenwasser reported that minor T-allele of rs2243250 was related to an elevated IL-4 activity by greater binding to nuclear transcriptional factor than C allele [22]. Therefore, we hypothesized cautiously according to allele frequency distribution in Chinese that the rare T allele of rs2243250 may influence genetic susceptibility to CAD or severity of the disease *via* modifying the IL-4 activity in Chinese Han population.

Many previous studies have reported the association between rs2243250 polymorphism and risk of cardiovascular disease. However, they failed to reach consistent results [17,23-29]. Mahmoudi et al. reported that the IL-4 rs2243250 polymorphism and IL-4 haplotypes were significantly associated with risk of ischemic heart failure in Iranian [25]. However, Chou et al. revealed lack of association between IL-4 polymorphisms and rheumatic heart disease [23]. The possible explanation for the inconsistent results arises from these following aspects: rs2243250 polymorphism may exert diverse effects in the pathogenesis of cardiovascular disease in different populations; cardiovascular diseases are multi-

factorial and gene-environment and gene-gene interactions may influence the disease susceptibility.

We also found that rs2243270 is in the promoter region of IL-4 and we observed that the GG genotype was associated with an increased risk of CAD. Only two previous studies reported the association between rs2243270 and disease susceptibility [29,30]. Kim et al. performed a study in Korean population on the association between IL-4 SNPs and asthmatics, and they found an association of IL-4 rs2243250 and rs2070874 with aspirin intolerance, but rs2243270 was not associated with aspirin hypersensitivity [29]. Rong et al. found that rs2243270 was associated with RCC and had a significant decreased risk of RCC [30].

Two limitations should be mentioned in the present study. First, since patients and controls were selected from only one hospital of China, the selection samples may not represent Han Chinese in other places, and the selection bias may be occurred. Second, the small sample size was quite small, resulting in a low statistical power to find differences between the patients and controls.

In conclusion, this study provides a new insight in CAD that rs2242250 and rs2243270 in the promoter region of IL-4 are positively associated with the risk of CAD in Han Chinese individuals. Our study suggests that IL-4 may be a useful biomarker for prediction of the susceptibility of this study.

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