Int J Clin Exp Pathol 2015;8(9):11653-11658
www.ijcep.com /ISSN:1936-2625/IJCEP0011843

Original Article

Association between interleukin-17 gene polymorphisms and risk of coronary artery disease

Lian Shuang[1,2], Zhiliang Li[1], Fengying Chen[2], Xiaoying Cui[2], Yuzhen Ning[2], Youle Su[3], Mei Dong[4]

[1]*Department of Cardiovascular, Zhujiang Hospital, Southern Medical University, Guangzhou 510280, China;*
[2]*Department of Emergency, Affiliated Hospital of Inner Mongolia Medical University, Hohhot 010051, China;*
[3]*Department of Neurosurgery, Affiliated Hospital of Inner Mongolia Medical University, Hohhot 010051, China;*
[4]*Department of Orthopedic Medicine, The Second Affiliated Hospital of Inner Mongolia Medical University, Hohhot 010030, China*

Received June 23, 2015; Accepted July 27, 2015; Epub September 1, 2015; Published September 15, 2015

Abstract: We conducted a case-control study to estimate the association between IL-17A rs2275913, rs3819025 and rs3748067 polymorphisms and development of coronary artery disease. A total of 415 patients with coronary artery disease and 448 health controls were recruited during the period of March 2013 and October 2014. Genotyping of IL-17A rs2275913, rs3819025 and rs3748067 were analyzed by polymerase chain reaction coupled with restriction fragment length polymorphism. By logistic regression analysis, we found that individuals with the AA genotype (OR, 2.18; 95% CI, 1.35-3.56) and the GA+AA genotype (OR, 1.39, 95% CI, 1.06-1.84) of rs2275913 were associated with an increased risk of coronary artery disease when compared with the GG genotype. Individuals carrying the GA+AA genotype of rs2275913 were more likely to have a higher risk of coronary artery disease in those with hypertension and smoking habit, and the adjusted ORs (95% CI) were 3.92 (2.13-6.82) and 2.74 (1.71-4.40). In conclusion, we suggest that individuals with the AA genotype and the GA+AA genotype of rs2275913 are associated with an increased risk of coronary artery disease, especially in those with hypertension and smoking habit.

Keywords: Interleukin-17, polymorphism, coronary artery disease

Introduction

Coronary artery disease is one of the main causes of morbidity and mortality worldwide, and more than 80% of the coronary artery diseases occur in low-to-middle income countries [1]. The etiology of coronary artery disease is not well understood, and the process of coronary artery disease is caused by many environmental factors, such as male gender, hypertension, smoking, drinking and diabetes mellitus as well as high serum cholesterol [1, 2]. However, not all of the individuals who exposed to similar environmental factors of coronary artery disease would suffer from coronary artery disease, which suggests that genetic factors have an important role in the susceptibility to coronary artery disease.

It is well known that inflammation plays an important role in the development of atherosclerosis [3]. Interleukin-17 (IL-17) is a novel family cytokine that consists of six protein members (from IL-17A to IL-17F), which plays an important role in many chronic inflammatory diseases [4, 5]. IL-17 is always produced by activated CD4+ T cells (Th17 cells) and other leukocytes, such as T cells, natural killer cells (NK cells), lymphoid tissue inducer-like cells (LTi-like cells), and neutrophils [6]. IL-17A is the most important member of IL-17, and it locates in 6q12 and constitute by three exons and two introns. IL-17A rs2275913, rs3819025 and rs3748067 are three common SNPs, and two previous studies have reported the association between IL-17 gene polymorphisms and risk of cardiovascular disease [7, 8], but the results are inconsistent. Therefore, we conducted a case-control study to estimate the association between IL-17A rs2275913, rs3819025 and rs3748067 polymorphisms and development of coronary artery disease.

Table 1. Characteristics of patients with coronary artery disease and control subjects

	CAD cases N = 415	%	Controls N = 448	%	χ^2 test or *t* test	*P* value
Mean age, years	59.20±10.85		60.10±10.35		1.25	0.11
<60	225	54.22	215	47.99	11.9	
≥60	190	45.78	233	52.01	3.34	0.07
Sex						
Male	321	77.35	265	59.15		
Female	94	22.65	183	40.85	11.65	0.001
BMI, kg/m^2	23.74±3.20		23.47±3.12		1.25	0.11
<24	285	68.67	246	54.91		
≥24	130	31.33	102	22.77	0.36	0.55
Hypertension						
No	283	68.19	352	78.57		
Yes	132	31.81	96	21.43	11.93	0.001
Diabetes mellitus						
No	305	73.49	384	85.71		
Yes	110	26.51	64	14.29	19.98	<0.001
Alcohol drinking						
Non-drinkers	181	43.61	247	55.13		
Drinkers	234	56.39	201	44.87	11.44	0.001
Tobacco smoking						
Non-smokers	233	56.14	295	65.85		
Smokers	182	43.86	153	34.15	8.54	0.003
TC, mmol/dL	198.40±45.75		174.65±32.50		8.84	<0.001
LDL-c, mmol/dL	117.52±26.55		97.42±23.64		11.76	<0.001
HDL-c, mmol/dL	37.35±9.10		44.25±8.25		11.68	<0.001
TG, mmol/dL	137.41±38.50		117.66±29.75		8.47	<0.001

Materials and methods

Subjects

A total of 462 patients with coronary artery disease were recruited during the period of March 2013 and October 2014 in the Affiliated Hospital of Inner Mongolia Medical University. All the cases were newly diagnosed by angiography, and the coronary artery disease was defined as a diameter stenosis of 50% in any of the main coronary arteries, including left main, left anterior descending, left circumflex artery or right coronary artery. The exclusion criteria were subjects who had myocardial spasms or a myocardial bridge, congenital heart disease, peripheral artery disease, autoimmune-related disease and renal as well as liver deceases or cancers. Finally, 415 patients were included into our study, with a participation rate of 89.83%.

Control subjects were randomly recruited from individuals who received a regular health-check up in our hospital during the same period. All the control subjects were diagnosed to have no history of coronary artery disease, atherosclerosis, had myocardial spasms or a myocardial bridge, congenital heart disease, peripheral artery disease, autoimmune-related disease and renal diseases. A total of 448 control subjects were collected into the Affiliated Hospital of Inner Mongolia Medical University and the Second Affiliated Hospital of Inner Mongolia Medical University.

The social-demographic characteristics were collected from patients with coronary artery disease and control subjects using a standardized questionnaire, such as age, gender, body mass index, hypertension, diabetes mellitus, alcohol drinking and tobacco smoking. Smoking status was based on self-reported smoking,

Table 2. Genotype distributions of IL-17A rs2275913, rs3819025 and rs3748067 and their association with risk of coronary artery disease

IL-17 gene	Base change	Patients	%	Controls	%	*P* value for HWE	OR (95% CI)[1]	*P* value
rs2275913								
GG		168	40.48	220	49.11		1.0 (Ref.)	-
GA		187	45.06	196	43.75		1.25 (0.93-1.68)	0.12
AA	G>A	60	14.46	36	8.04	0.4	2.18 (1.35-3.56)	0.001
GA+AA		247	59.52	232	51.79		1.39 (1.06-1.84)	0.02
rs3819025								
AA		179	43.13	212	47.32		1.0 (Ref.)	-
AG		177	42.65	180	40.18		1.16 (0.86-1.57)	0.3
GG	A>G	59	14.22	56	12.50	0.07	1.25 (0.81-1.93)	0.29
AG+GG		236	56.87	236	52.68		1.18 (0.90-1.56)	0.22
rs3748067								
TT		301	72.53	339	75.67		1.0 (Ref.)	-
TC		91	21.93	95	21.21		1.08 (0.77-1.52)	0.65
CC	C>T	23	5.54	14	3.13	0.03	1.85 (0.89-3.96)	0.07
TC+CC		114	27.47	109	24.33		1.18 (0.86-1.62)	0.29

[1]Adjusted for sex, age, hypertension, diabetes mellitus, alcohol drinking, tobacco smoking, TC, LDL-c, HDL-c and TG.

and the subjects who had never smoked less than 100 cigarettes in their lives were classified as non-smokers. Drinking status was defined as non-drinker and drinker. The clinical data were collected from medical records, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c) and triglyceride (TG).

A written informed consent was gained from each subject before entering the study group. The collection of blood samples for this study was previously approved by ethics committee of our hospital.

Genetic analysis

Each participant was asked to provide a 5-ml peripheral venous blood sample after enrolling into our study. The DNA was extracted from peripheral blood by salt extraction. Genotyping of IL-17A rs2275913, rs3819025 and rs3748067 was analyzed by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). The primers for IL-17A rs2275913, rs3819025 and rs3748067 were as follows: rs2275913, 5'-GCAGCTCTGCTCAGCTTCTAA-3' (forward) and 5'-TTCAGGGGTGACACCATTTT-3' (reverse); rs3819025, 5'-GGTGTCACCCCTGAACCCACT-3' (forward) and 5'-CATGCCCACGGTCCAGAAATA-3' (reverse); rs3748067, 5'-AAGCAGGGAGCCTGCAGAGTG-3' (forward) and 5'-GGCACCACACAACCCAGAAAG-3' (reverse). The PCR fragments of the investigated polymorphisms were subsequently digested with their specific restriction enzyme, and *Bst*ENI for rs3748067C>T, *Mae*II for rs3819025 and *Ava*II for rs2275913. The amplification conditions were 95°C for 5 min, then 30 cycles of 94°C for 0.5 min, 60°C for 0.5 min and 72°C for 1 min, at last 72°C for 10 min. Digestion products were separated by electrophoresis on ethidium bromide stained agarose gel and visualized under UV light.

Statistical analysis

All the statistical analyses were conducted with the SPSS 16.0 statistical software (SPSS, Chicago, IL). The distributions of demographic and clinical characteristics of patients with coronary artery disease and control subjects were compared by Pearson χ^2 test or student t test. The distribution of genotypes in controls was tested for deviation from Hardy-Weinberg equilibrium. Multiple logistic regression models were established to estimate relative risks of IL-17A rs2275913, rs3819025 and rs3748067 for coronary artery disease after adjustment for confounding factors. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calcu-

Table 3. Interaction between rs2275913 polymorphism and demographic characteristics of subjects in the risk of coronary artery disease

Variables	rs2275913				OR (95% CI)[1]	*P* value
	TT		TC+CC			
	Case	Control	Case	Control		
Hypertension						
No	125	184	158	168	1.34 (0.94-1.85)	0.07
Yes	43	63	89	33	3.92 (2.13-6.82)	<0.001
Diabetes mellitus						
No	123	210	182	174	1.75 (0.96-2.13)	0.06
Yes	45	37	65	27	1.85 (0.94-4.10)	0.05
Alcohol drinking						
Non-drinkers	75	135	106	112	1.71 (0.92-2.05)	0.08
Drinkers	93	112	141	89	1.69 (0.81-2.48)	0.1
Tobacco smoking						
Non-smokers	112	163	121	132	1.33 (0.93-1.91)	0.1
Smokers	56	84	126	69	2.74 (1.71-4.40)	<0.001

[1]Adjusted for sex and age.

lated. All tests were two-sided with a significant level of *P*-value <0.05.

Results

The characteristics of patients with coronary artery disease and control subjects were shown in **Table 1**. No significant difference was found in age between patients with coronary artery disease and control subjects, and the mean ages of patients with coronary artery disease and control subjects were 59.20±10.85 and 60.10±10.35 years, respectively (*P*>0.05). By χ^2 test, we found that patients with coronary artery disease were more likely to be males, suffer from hypertension and diabetes, and be smokers and drinkers (*P*<0.05). Moreover, patients with coronary artery disease had higher TC, LDL-c and TG and have lower HDL-c when compared with control subjects (*P*<0.05).

The genotype distribution of rs2275913 and rs3819025 confirmed with Hardy-Weinberg equilibrium in the control group (*P* values were 0.40 and 0.07, respectively), but rs3748067 were not (*P* value for HWE was 0.03). By χ^2 test, the genotype distributions of rs2275913 was significant different between patients with coronary artery disease and control subjects (χ^2 = 11.62, *P* value = 0.003). By logistic regression analysis, we found that individuals with the AA genotype and the GA+AA genotype of rs2275913 were associated with an increased risk of coronary artery disease when compared with the GG genotype, and the adjusted ORs (95% CI) were 2.18 (1.35-3.56) and 1.39 (1.06-1.84) for the AA genotype and the GA+AA genotype, respectively (**Table 2**). However, no significant association was found between rs3819025 and rs3748067 polymorphisms and risk of coronary artery disease.

We also conducted gene-environmental analysis to investigate the association between rs2275913 polymorphism and demographic characteristics of subjects in the risk of coronary artery disease. We found individuals carrying the GA+AA genotype of rs2275913 were more likely to have a higher risk of coronary artery disease in those with hypertension and smoking habit, and the adjusted ORs (95% CI) were 3.92 (2.13-6.82) and 2.74 (1.71-4.40), respectively (**Table 3**). However, we did not find significant association of rs2275913 polymorphism with diabetes mellitus and alcohol drinking in the development of coronary artery disease (*P* value >0.05).

Discussion

It is well known that genetic susceptibility to cardiovascular disease has gain an increasing attention to investigate the polymorphisms of genes involved in the development of coronary artery disease. Inflammation and related cytokines are involved in both innate and acquired

immune responses, and play an important role in the inflammatory response that contributes to the development of cardiovascular disease.

IL-17A is a relatively novel cytokine family contains six homologous members, and IL-17A is an important inflammatory cytokine to connect the innate and adaptive immunity [9]. Molecular studies have shown that IL-17 is an essential proinflammatory cytokine to evoke lots of cytokines and chemokines secretion through different cell types, such as mesenchymal cells and myeloid cells to promote monocytes and neutrophils into the microenvironment of inflammation [10].

Currently, many epidemiological studies have investigated the association between IL-17 gene and development of cardiovascular and cerebrovascular diseases [11-14]. Lv et al. conducted an experimental study, and reported that IL-17 could cause inflammatory immune responses and neuronal damage, such as cerebral ischemia/reperfusion injury [11]. Li et al. reported that Th17/Treg cells were imbalanced in atherosclerotic cerebral infarction patients, and contributed to the occurrence of atherosclerotic cerebral infarction, suggesting their pathogenetic role in atherosclerotic cerebral infarction [13]. Márquez et al. conducted a meta-analysis with 1266 biopsy-proven giant cell arteritis patients and 3779 healthy controls, and they reported that IL-17 rs2275913 and rs4711998 polymorphisms were associated with increased risk of giant cell arteritis [14].

For the association between IL-17 gene polymorphisms and development of cardiovascular diseases, only two previous studies reported their association [15, 16]. Pei et al. conducted a case-control association in a Chinese population, and they have indicated that IL-17F rs763780 polymorphism is unlikely to contribute to the development of myocardial infarction [15]. Another study also conducted a case-control study in a Chinese population, and has reported that IL-17A rs8193037 is associated with an increased risk of coronary artery disease and contributes to increased expression of IL-17A in acute myocardial infarction [16]. In our study, we found that individuals with the AA genotype and the GA+AA genotype of rs2275913 were associated with an increased risk of coronary artery disease when compared with TT genotype, which is inconsistent with the results of previous studies. The discrepancies of these results may be caused by differences in ethnicities, study design, and sample size as well as by chance.

Moreover, our study found that individuals carrying the GA+AA genotype of rs2275913 were more likely to have a higher risk of coronary artery disease in smokers. Previous studies reported that IL-17 gene polymorphism was associated with smoking related diseases [17-19]. Further studies are greatly needed to confirm our study.

In conclusion, we suggest that individuals with the AA genotype and the GA+AA genotype of rs2275913 were associated with an increased risk of coronary artery disease, especially in those with hypertension and smoking habit. Further genetic studies with large sample size are greatly needed to confirm the association between IL-17 gene polymorphisms and risk of coronary artery disease.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhiliang Li, Department of Cardiovascular, Zhujiang Hospital, Southern Medical University, Guangzhou 510280, China. Tel: +86-20-62783688; Fax: +86-20-62783688; E-mail: lizhouxiaoyu@163.com

References

[1] Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JM, Kushner FG, Lauer MS, Shaw LJ, Smith SC Jr, Taylor AJ, Weintraub WS, Wenger NK, Jacobs AK, Smith SC Jr, Anderson JL, Albert N, Buller CE, Creager MA, Ettinger SM, Guyton RA, Halperin JL, Hochman JS, Kushner FG, Nishimura R, Ohman EM, Page RL, Stevenson WG, Tarkington LG, Yancy CW; American College of Cardiology Foundation; American Heart Association. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2010; 56: e50-103.

[2] Libby P. What have we learned about the biology of atherosclerosis? The role of inflammation. Am J Cardiol 2001; 88: 3J-6J.

[3] Weber C, Hristov M. Atherogenesis and inflammation. From cellular mediators to regulatory mechanisms of inflammation in atherosclerosis. Hamostaseologie 2015; 35: 99-101.
[4] Cătană CS, Berindan Neagoe I, Cozma V, Magdaş C, Tăbăran F, Dumitraşcu DL. Contribution of the IL-17/IL-23 axis to the pathogenesis of inflammatory bowel disease. World J Gastroenterol 2015; 21: 5823-5830.
[5] Isailovic N, Daigo K, Mantovani A, Selmi C. Interleukin-17 and innate immunity in infections and chronic inflammation. J Autoimmun 2015; 60: 1-11.
[6] Cua DJ, Tato CM. Innate il-17-producing cells: The sentinels of the immune system. Nat Rev Immunol 2010; 10: 479-489.
[7] Pei F, Han Y, Zhang X, Yan C, Huang M, Deng J, Kang J. Association analysis of the IL-17F His161Arg polymorphism in myocardial infarction. Coron Artery Dis 2009; 20: 513-517.
[8] Zhang X, Pei F, Zhang M, Yan C, Huang M, Wang T, Han Y. Interleukin-17A gene variants and risk of coronary artery disease: a large angiography-based study. Clin Chim Acta 2011; 412: 327-331.
[9] Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity 2004; 21: 467-476.
[10] Iwakura Y, Ishigame H, Saijo S, Nakae S. Functional specialization of interleukin-17 family members. Immunity 2011; 34: 149-162.
[11] Lv M, Liu Y, Zhang J, Sun L, Liu Z, Zhang S, Wang B, Su D, Su Z. Roles of inflammation response in microglia cell through Toll-like receptors 2/interleukin-23/interleukin-17 pathway in cerebral ischemia/reperfusion injury. Neuroscience 2011; 176: 162-172.
[12] Zeng L, Wang Y, Liu J, Wang L, Weng S, Chen K, Domino EF, Yang GY. Pro-inflammatory cytokine network in peripheral inflammation response to cerebral ischemia. Neurosci Lett 2013; 548: 4-9.
[13] Li Q, Wang Y, Yu F, Wang YM, Zhang C, Hu C, Wu Z, Xu X, Hu S. Peripheral Th17/Treg imbalance in patients with atherosclerotic cerebral infarction. Int J Clin Exp Pathol 2013; 6: 1015-1027.
[14] Márquez A, Hernández-Rodríguez J, Cid MC, Solans R, Castañeda S, Fernández-Contreras ME, Ramentol M, Morado IC, Narváez J, Gómez-Vaquero C, Martínez-Taboada VM, Ortego-Centeno N, Sopeña B, Monfort J, García-Villanueva MJ, Caminal-Montero L, de Miguel E, Blanco R; Spanish GCA Consortium, Palm O, Molberg O, Latus J, Braun N, Moosig F, Witte T, Beretta L, Santaniello A, Pazzola G, Boiardi L, Salvarani C, González-Gay MA, Martín J. Influence of the IL17A locus in giant cell arteritis susceptibility. Ann Rheum Dis 2014; 73: 1742-1745.
[15] Pei F, Han Y, Zhang X, Yan C, Huang M, Deng J, Kang J. Association analysis of the IL-17F His161Arg polymorphism in myocardial infarction. Coron Artery Dis 2009; 20: 513-517.
[16] Zhang X, Pei F, Zhang M, Yan C, Huang M, Wang T, Han Y. Interleukin-17A gene variants and risk of coronary artery disease: a large angiography-based study. Clin Chim Acta 2011; 412: 327-331.
[17] Chang Y, Al-Alwan L, Audusseau S, Chouiali F, Carlevaro-Fita J, Iwakura Y, Baglole CJ, Eidelman DH, Hamid Q. Genetic deletion of IL-17A reduces cigarette smoke-induced inflammation and alveolar type II cell apoptosis. Am J Physiol Lung Cell Mol Physiol 2014; 306: L132-L143.
[18] Belchamber K, Hall DA, Hourani SM. Smoking enhances the proinflammatory effects of nucleotides on cytokine release from human lung. PLoS One 2014; 9: e99711.
[19] Li N, Zhang C, Chen Z, Bai L, Nie M, Zhou B, Xu H. Interleukin 17A and interleukin 17F polymorphisms are associated with oral squamous cell carcinoma susceptibility in a Chinese population. J Oral Maxillofac Surg 2015; 73: 267-273.