Int J Clin Exp Pathol 2016;9(2):2319-2324
www.ijcep.com /ISSN:1936-2625/IJCEP0019594

Original Article

IL-17F rs763780T>C polymorphism contributes to the development of coronary artery disease in a Chinese population

Yi Dang[1], Yingxiao Li[1], Xiuru Ma[2], Yuetao Xie[1], Qianhui Zhang[1], Kexin Yuan[1], Xiaoyong Qi[1]

[1]Department of Cardiology, Hebei General Hospital, Shijiazhuang, China; [2]College of Integrated Chinese and Western Medicine, Hebei University of Chinese Medicine, Shijiazhuang, China

Received November 11, 2015; Accepted January 12, 2016; Epub February 1, 2016; Published February 15, 2016

Abstract: We conducted a case-control study to investigate the role of *IL-17A* (rs2275913G>A and rs3748067C>T) and *IL-17F* (rs763780 T>C) gene polymorphisms in the susceptibility to CAD in a Chinese population. Between July 2013 and December 2014, a total of 184 patients with CAD and 184 control subjects were consecutively selected from the Hebei General Hospital. The *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C polymorphisms were genotyped using PCR-RFLP. By chi-square test, a significant different was found in the genotype distributions of *IL-17A* rs2275913G>A between CAD patients and control subjects (χ^2=0.66, *P*=0.72). By unconditional multiple logistic regression analysis, we found that the CC and TC+CC genotypes of *IL-17F* rs763780T>C was associated with increased risk of CAD when compared with the TT genotype, and the adjusted OR (95% CI) was 3.55 (1.20-12.66) and 1.71 (1.03-2.97), respectively. However, no significant association was found between *IL-17A* rs2275913G>A and rs3748067C>T and development of CAD. In our study, we suggest that *IL-17F* rs763780T>C polymorphism could influence the development of CAD in a Chinese population.

Keywords: *IL-17A*, *IL-17F*, polymorphism, coronary artery disease

Introduction

Cardiovascular disease is one of the leading causes of mortality and loss of disability-adjusted life years in both developed and developing countries, and coronary artery disease (CAD) is the most common type of cardiovascular disease and caused by atherosclerosis [1]. It is well known that the susceptibility of CAD is involved in many complex factors, including many environmental and behavioral factors and their interactions, such as hypertension, hypercholesterolemia, diabetes, obesity, physical activity, high quantity of sugar dietary and tobacco smoking as well as alcohol drinking [2]. However, individuals have shown high individualized susceptibility to CAD even when they are exposure to the same risk factors of this disease, which suggests that inherited factors contribute to the development of coronary artery disease. A recent study has reported that the inherited factors account for about 50%% in the risk of coronary artery disease [3]. Inflammation plays a role in the development of atherosclerosis, including oxidative damage, cell proliferation, plaque evolution, and destabilization [4-7]. Moreover, it is reported that several inflammatory markers play an important role in the orchestration of atherogenesis; in addition, interleukins are considered to be involved in chronic vascular inflammatory response in atherosclerosis [8-10]. Interleukin-17 (IL-17), a novel family of cytokines consisting of six protein members (from *IL-17A* to *IL-17F*), plays a pivotal role in many chronic inflammatory diseases. *IL-17A* and *IL-17F* are the most important members of the family, and they are located in chromosome 6q12 with three exons and two introns. Only three previous studies have reported the association between *IL-17* gene polymorphisms and development of CAD, but the results are in conclusive [11-13]. We conducted a case-control study to investigate the role of *IL-17A* (rs2275913G>A and rs3748067C>T) and *IL-17F* (rs763780 T>C) gene polymorphisms in the susceptibility to CAD in a Chinese population.

Materials and methods

Patients

Between July 2013 and December 2014, a total of 184 patients with CAD were consecutively selected from the Hebei General Hospital. All the CAD patients received coronary angiography according to a structured method. The CAD was independently diagnosed by two cardiologists. All CAD was defined as the presence of at least one main coronary arteries of more than 50% fifty luminal diameter based on coronary angiography, or a history of prior angioplasty, coronary artery bypass surgery or an MI history validated by on typical electrocardiographic changes as well as increased in the plasma activities of enzymes. The findings of coronary angiography were independently assessed by two experienced imaging specialists.

A total of 184 controls were selected from subjects who went to our hospital for a health examination. All the control subjects were diagnosed to be free of CAD by coronary angiography examination, and they were confirmed to have no history of arteriosclerotic lesions. The exclusion criteria of patients and controls that had a myocardial bridge, congenital heart disease, childhood hypertension, and serious kidney or liver diseases as well as malignant tumors were excluded from our study. Each control was matched with one patient by sex and age (±5 years).

The traditional coronary risk factors were collected from medical records and a self-designed questionnaire. The collected information included hypertension, diabetes mellitus, tobacco smoking and alcohol drinking, body mass indexes, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c). The blood urea nitrogen, TC, TG, LDL-c and HDL-c were determined through the fasting blood samples. 5 mL blood sample was obtained from each patient and control prior to participating into this study, and a signed informed consent form was obtained from all patients and controls before their participation in the study. The protocol of this project was approved by the Ethics Committee of the Hebei General Hospital.

DNA extraction and genotyping analysis

Five mL blood sample was taken to perform DNA extraction using the TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer instructions. The *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C polymorphism were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The forward and reverse primers for *IL-17A* rs2275913G>A were 5'-GCCCTTCCCATTTTCCTTCAGA-3' and 5'-CCAATCAACTGGGGATGGATGA-3', respectively. The forward and reverse primers for *IL-17A* rs3748067C>T were 5'-AAGCAGGGAGCCTGCAGAGTG-3' and 5'-GGCACCACACAACCCAGAAAG-3', respectively. The forward and reverse primers for *IL-17F* rs763780T>C were 5'-CTGTTTCCATCCGTGCAGGTC-3' and 5'-TGGTGACTGTTGGCTGCACCT-3', respectively. The product sizes for *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C were 210 bp, 217 bp and 188 bp, respectively. The PCR fragments of the investigated polymorphisms were subsequently digested with their specific restriction enzyme. The PCR reaction conditions were as follows: an initial denaturation at 94°C for 5 min, then 30 cycles of denaturation at 94°C for 60 sec, annealing at 55°C for 60 sec, and extension at 72°C for 2 min, followed by a final extension step at 72°C for 5 min. The amplified products were determined using electrophoresis in a 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

Statistical analysis

Statistically significant differences in traditional coronary risk factors of CAD between CAD patients and control subjects were compared by the Chi-square (χ^2) test and student T test. The differences in the genotype distributions of *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C between CAD patients and control subjects were compared using χ^2 test. Whether the genotype distribution of *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C were deviated from Hardy-Weinberg equilibrium (HWE) in controls were assessed using the goodness-of-fit χ^2-test. The minor allele frequencies (MAFs) of *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C were calculated, which are com-

Table 1. Demographic and clinical characteristics of study subjects

	CAD patients N=184	%	Controls N=184	%	χ^2 test or *t* test	*P* value
Mean age, years	56.51±10.62		57.53±12.15		0.86	0.19
Sex						
Male	131	71.20	131	71.20		
Female	53	28.80	53	28.80	0.00	1.00
BMI, kg/m^2	25.32±2.53		23.55±2.67		6.53	<0.001
Hypertension						
No	100	54.35	117	63.59		
Yes	84	45.65	67	36.41	3.25	0.07
Diabetes mellitus						
No	150	81.52	159	86.41		
Yes	34	18.48	25	13.59	1.64	0.20
Alcohol drinking						
Never	102	55.43	117	63.59		
Current or ever	82	44.57	67	36.41	2.54	0.11
Tobacco smoking						
Never	110	59.78	130	70.65		
Current or ever	74	40.22	54	29.35	4.79	0.03
TC, mmol/L	4.74±1.03		4.55±0.92		1.87	0.03
TG, mmol/L	2.43±0.66		2.09±1.18		3.41	<0.001
LDL-c, mmol/L	2.35±0.41		2.32±0.45		0.67	0.25
HDL-c, mmol/L	1.29±0.24		1.24±0.30		1.76	0.04

pared with the MAFs in the National Center for Biotechnology Information SNP database. Using conditional logistic regression analysis, the association between *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C and the development of CAD were estimated, and the results were expressed using Odd's ratio (OR) and 95% confidence interval (95% CI). The major homozygous genotypes of the *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C were taken as reference group. Statistical analysis was conducted using the SPSS 17.0 (SPSS Inc., Chicago, IL, USA), and $P<0.05$ was considered as significant difference.

Results

The lifestyle and clinical characteristics of patients with CAD and control subjects were shown in **Table 1**. The mean ages of CAD patients and control subjects were 56.51±10.62 and 57.53±12.15 years, respectively. As expected, no significant different was observed between CAD patients and control subjects in terms of sex and age. Compared to the control subjects, CAD patients were more likely to have higher BMI ($t=6.53$, $P<0.001$), have a habit of tobacco smoking ($\chi^2=4.79$, $P=0.03$), and have higher levels of TC ($\chi^2=1.87$, $P=0.03$), TG ($\chi^2=3.41$, $P<0.001$) and HDL-c ($t=1.76$, $P=0.04$). However, there was no significant difference in diabetes mellitus ($\chi^2=1.64$, $P=0.20$), alcohol drinking ($\chi^2=2.54$, $P=0.11$) and LDL-c ($t=0.67$, $P=0.25$) between CAD patients and control subjects.

Genotype distributions of *IL-17A* rs2275913G->A and rs3748067C>T and *IL-17F* rs763780T->C gene polymorphisms were shown in **Table 2**. The genotype distributions of *IL-17A* rs2275913G>A in the control subjects confirmed with the Hardy-Weinberg equilibrium (*P* value for HWE was 0.64), but those of *IL-17A* rs3748067C>T and *IL-17F* rs763780T>C did not. By chi-square test, a significant different was found in the genotype distributions of *IL-17A* rs2275913G>A between CAD patients and control subjects ($\chi^2=0.66$, $P=0.72$). However, no significant difference was found in the genotype distributions of *IL-17A* rs3748067C>T ($\chi^2=3.02$, $P=0.22$) and *IL-17F* rs763780T>C ($\chi^2=6.83$, $P=0.03$) between the

Table 2. Genotype distributions of *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C between patients and control subjects

IL-17	Patients	%	Controls	%	χ^2 test	*P* value	*P for HWE*	Minor allele frequency	
	N=184		N=184				In controls	In NCBI database	In controls
rs2275913G>A									
GG	82	44.57	89	48.37					
GA	81	44.02	76	41.30					
AA	22	11.96	19	10.33	0.66	0.72	0.64	0.2927	0.3098
rs3748067C>T									
CC	148	80.43	157	85.33					
CT	26	14.13	23	12.50					
TT	10	5.43	4	2.17	3.02	0.22	0.01	0.0769	0.0842
rs763780T>C									
TT	139	75.54	154	83.70					
TC	29	15.76	25	13.59					
CC	16	8.70	5	2.72	6.83	0.03	0.004	0.0935	0.0951

Table 3. Association between *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C and CAD risk

IL-17	Patients	%	Controls	%	OR (95% CI)[1]	*P* value
	N=184		N=184			
rs2275913G>A						
GG	82	44.57	89	48.37	1.0 (Ref.)	-
GA	81	44.02	76	41.30	1.16 (0.73-1.83)	0.51
AA	22	11.96	19	10.33	1.26 (0.60-2.65)	0.51
GA+AA	103	55.98	95	51.63	1.18 (0.77-1.81)	0.44
rs3748067C>T						
CC	148	80.43	157	85.33	1.0 (Ref.)	-
CT	26	14.13	23	12.50	1.20 (0.63-2.31)	0.56
TT	10	5.43	4	2.17	2.65 (0.74-11.80)	0.09
CT+TT	36	19.57	27	14.67	1.41 (0.79-2.55)	0.21
rs763780T>C						
TT	139	75.54	154	83.70	1.0 (Ref.)	-
TC	29	15.76	25	13.59	1.29 (0.69-2.41)	0.40
CC	16	8.70	5	2.72	3.55 (1.20-12.66)	0.01
TC+CC	45	24.46	30	16.30	1.71 (1.03-2.97)	0.04

[1]Adjusted for BMI, tobacco smoking, TC and TG and HDL-c.

patients and controls. The minor allele frequencies of *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C were similar with those in the National Centre for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/snp).

By unconditional multiple logistic regression analysis, we found that the CC genotype of rs763780T>C was associated with increased risk of CAD when compared with the TT genotype, and the adjusted OR (95% CI) was 3.55 (1.20-12.66) (**Table 3**). Moreover, the TC+CC genotype of *IL-17F* rs763780T>C was correlated with an elevated risk of CAD compared to the TT genotype, and the adjusted OR (95% CI) was 1.71 (1.03-2.97). However, no significant association was found between *IL-17A* rs2275913G>A and rs3748067C>T polymorphisms and development of CAD.

Discussion

Genetic susceptibility to diseases has attracted increasing attention to the study of gene poly-

morphisms involved in the development of several kinds of diseases, such as cardiovascular disease and malignant tumor. Inflammation and related cytokines contribute to the atherosclerotic developments, such as oxidative damage, cell proliferation and plaque evolution and destabilization [4-7]. Previous studies have shown that inflammatory state play an important role in orchestration of atherogenesis [14-16]. Interleukins are regarded as an important role in chronic vascular inflammatory response that is typical of atherosclerosis [8-10]. In our study, we conducted a study to assess the role of *IL-17A* rs2275913G>A and rs3748067C>T and *IL-17F* rs763780T>C gene polymoprhisms in the development of CAD, and we revealed that the CC and TC+CC genotypes of *IL-17F* rs763780T>C were correlated with an increased risk of CAD.

IL-17 is a novel cytokine family consisting of six homologous members (from *IL-17A* to *IL-17F*), and it contributes to connecting innate and adaptive immunity [17]. Epidemiologic studies have suggested that *IL-17* polymorphisms were associated with an essential proinflammatory cytokine that evokes cytokine and chemokine secretion through different cell types, such as mesenchymal cells and myeloid cells, to recruit monocytes and neutrophils into the inflammatory microenvironment [18]. Furthermore, *IL-17* promotes the expression of antimicrobial peptides and facilitates host defense mechanism against infections [19, 20].

Previous studies have reported that *IL-17* gene was associated with different kinds of diseases, such as cancers, stroke and carotid atherosclerosis [11, 21-24]. Hu et al. suggested that the increase in *IL-17A*-producing cells and decrease in Treg cells might contribute to the pathogenesis of ischemic stroke [21]. Abbas et al. have shown an association between *IL-17* gene polymorphisms and development of carotid atherosclerosis [22]. Wang et al. conducted a meta-analysis with nine case-control studies, and they reported that *IL-17A* and *IL-17F* genotypes were associated with increased risk of cancer, especially for gastric cancer [23]. Erbel et al. have indicated that *IL-17A* prevents atherosclerotic lesion progression and induces plaque stabilization in advanced lesions in mice [24]. Vargas-Alarcón et al. suggest that *IL-17A* haplotypes are involved in the risk of developing premature CAD and cardiovascular risk factors in Mexican individuals [11].

For the correlation between *IL-17* gene polymorphisms and development of CAD, only three studies have reported their association [11-13]. Vargas-Alarcón et al. have suggested that IL-17A polymorphisms contribute to the development of CAD [11]. Zhang et al. conducted a case-control study with 1031 CAD patients and 935 control subjects, and they have reported that IL-17A rs8193037 G allele is associated with increased expression of IL-17A and correlated with risk of CAD in a Chinese population [12]. Pei et al. have conducted a case-control study with 513 unrelated myocardial infarction and 477 controls in a Chinese population, and they have indicated that IL-17F His161Arg (rs763780) is unlikely to be a major contributor to the development of myocardial infarction [13]. However, our study found that *IL-17F* rs763780T>C polymorphism was associated with an increased risk of CAD. The discrepancies between studies may be caused by different ethnicities, selection of patients and controls and sample size. Further studies with large sample size are greatly needed to confirm the findings of our study.

In our study, we suggest that *IL-17F* rs763780T>C polymorphism could influence the development of CAD in a Chinese population. Future studies with larger sample sizes may help elucidate the impact of IL-17 gene polymorphisms on the risk of CAD.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiaoyong Qi, Department of Cardiology, Hebei General Hospital, 348 Heping West Road, Shijiazhuang 050051, China. Tel: +86-311-85988937; Fax: +86-311-85988937; E-mail: xiaoyongqi66@sina.com

References

[1] Ross R. Atherosclerosis-an inflammatory disease. N Engl J Med 1999; 340: 115-26.

[2] Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. N Engl J Med 2000; 343: 1139-1147.

[3] Roberts R, Stewart AF. Genes and coronary artery disease: where are we? J Am Coll Cardiol 2012; 60: 1715-21.
[4] Buja LM, Willerson JT. Role of inflammation in coronary plaque disruption. Circulation 1994; 89: 503-5.
[5] Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ. Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. Circulation 1995; 91: 2488-96.
[6] Frangogiannis NG, Smith CW and Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res 2002; 53: 31-47.
[7] Mauriello A, Sangiorgi G, Fratoni S, Palmieri G, Bonanno E, Anemona L, Schwartz RS, Spagnoli LG. Diffuse and active inflammation occurs in both vulnerable and stable plaques of the entire coronary tree: a histopathologic study of patients dying of acute myocardial infarction. J Am Coll Cardiol 2005; 45: 1585-1893.
[8] von der Thusen JH, Kuiper J, van Berkel TJ, Biessen EA. Interleukins in atherosclerosis: molecular pathways and therapeutic potential. Pharmacol Rev 2003; 55: 133-66.
[9] Tziakas DN, Chalikias GK, Tentes IK, Stakos D, Chatzikyriakou SV, Mitrousi K, Kortsaris AX, Kaski JC, Boudoulas H. Interleukin-8 is increased in the membrane of circulating erythrocytes in patients with acute coronary syndrome. Eur Heart J 2008; 29: 2713-22.
[10] Aukrust P, Halvorsen B, Yndestad A, Ueland T, Øie E, Otterdal K, Gullestad L, Damås JK. Chemokines and cardiovascular risk. Arterioscler Thromb Vasc Biol 2008; 28: 1909-19.
[11] Vargas-Alarcón G, Angeles-Martínez J, Villarreal-Molina T, Alvarez-León E, Posadas-Sánchez R, Cardoso-Saldaña G, Ramírez-Bello J, Pérez-Hernández N, Juárez-Rojas JG, Rodríguez-Pérez JM, Fragoso JM, Posadas-Romero C. Interleukin-17A gene haplotypes are associated with risk of premature coronary artery disease in Mexican patients from the Genetics of Atherosclerotic Disease (GEA) study. PLoS One 2015; 10: e0114943.
[12] 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: 327-31.
[13] 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: 513-7.
[14] Maier W, Altwegg LA, Corti R, Gay S, Hersberger M, Maly FE, Sütsch G, Roffi M, Neidhart M, Eberli FR, Tanner FC, Gobbi S, von Eckardstein A, Lüscher TF. Inflammatory markers at the site of ruptured plaque in acute myocardial infarction: locally increased interleukin-6 and serum amyloid A but decreased C-reactive protein. Circulation 2005; 111: 1355-61.
[15] Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F; Centers for Disease Control and Prevention; American Heart Association. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003; 107: 499-511.
[16] Rosenson RS. Biomarkers, atherosclerosis and cardiovascular events. Expert Rev Cardiovasc Ther 2008; 6: 619-22.
[17] Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity 2004; 21: 467-76.
[18] Iwakura Y, Ishigame H, Saijo S, Nakae S. Functional specialization of interleukin-17 family members. Immunity 2011; 34: 149-62.
[19] Matsuzaki G, Umemura M. Interleukin-17 as an effector molecule of innate and acquired immunity against infections. Microbiol Immunol 2007; 51: 1139-47.
[20] Kao CY, Chen Y, Thai P, Wachi S, Huang F, Kim C, Harper RW, Wu R. IL-17 markedly up-regulates beta-defensin-2 expression in human airway epithelium via JAK and NF-kappaB signaling pathways. J Immunol 2004; 173: 3482-9135.
[21] Hu Y, Zheng Y, Wu Y, Ni B, Shi S. Imbalance between IL-17A-producing cells and regulatory T cells during ischemic stroke. Mediators Inflamm 2014; 2014: 813045.
[22] Abbas A, Gregersen I, Holm S, Daissormont I, Bjerkeli V, Krohg-Sørensen K, Skagen KR, Dahl TB, Russell D, Almås T, Bundgaard D, Alteheld LH, Rashidi A, Dahl CP, Michelsen AE, Biessen EA, Aukrust P, Halvorsen B, Skjelland M. Interleukin 23 levels are increased in carotid atherosclerosis: possible role for the interleukin 23/interleukin 17 axis. Stroke 2015; 46: 793-9.
[23] Wang H, Zhang Y, Liu Z, Zhang Y, Zhao H, Du S. The IL-17A G-197A and IL-17F 7488T/C polymorphisms are associated with increased risk of cancer in Asians: a meta-analysis. Drug Des Devel Ther 2015; 9: 5159-68.
[24] Erbel C, Akhavanpoor M, Okuyucu D, Wangler S, Dietz A, Zhao L, Stellos K, Little KM, Lasitschka F, Doesch A, Hakimi M, Dengler TJ, Giese T, Blessing E, Katus HA, Gleissner CA. IL-17A influences essential functions of the monocyte/macrophage lineage and is involved in advanced murine and human atherosclerosis. J Immunol 2014; 193: 4344-55.