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Original Article

IL-4 rs2243250 was associated with risk of coronary artery disease in a Chinese population

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Abstract: Coronary artery disease (CAD) is caused by coronary artery stenosis or obstruction, resulting in myocardial ischemia, hypoxia and leading to necrosis of the human heart. Several studies have shown that interleukin genes and its single nucleotide polymorphisms (SNPs) had the relation with this disease, but never referred to interleukin-4 (IL-4). In this study, we selected IL-4 SNPs rs2243250 (589 C/T), rs2227284 (107 T/C), rs2070874 (33 T/C) and IL-4R (IL-4 receptor) rs1801275 (576 Q/R) as the research targets. A case-control study on the relationship between IL-4 and its gene polymorphisms with CAD patients was performed, contained 420 cases and 420 normal people as control in a Chinese population. PCR (polymerase chain reaction) amplification of the genes and sequencing methods were used. SPSS17.0 software was used for statistical analysis of data. Alleles and genotypes of IL-4 rs2227284, rs2070874 and rs1801275 had no statistical significant difference between CAD patients group and control group ($P>0.05$), while IL-4 rs2243250 has statistical significant difference between two groups ($P<0.05$), showing that IL-4 (589 C/T) CT and TT genotype and T allele frequencies were significantly higher than those in the control group, OR value were 1.44 (1.05-1.89), 2.08 (1.41-3.31) and 1.52 (1.26-1.92). Logistic regression analysis showed that IL-4 (589 C/T) CT genotype and smoking related environment had positive interaction (OR=2.14, 95% CI: 1.37-3.62). IL-4 rs2243250 (589 C/T) allele was associated with coronary artery disease patients in Chinese population, and CT, TT genotype has a high risk of developing CAD; the CT genotypes with smoking related environment have positive interaction, increasing the risk of CAD.

Keywords: IL-4, polymorphism, coronary artery disease, environmental factors

Introduction

Coronary artery disease (CAD) is caused by coronary artery stenosis or obstruction, resulting in myocardial ischemia, hypoxia and leading to necrosis of the human heart [1, 2]. The pathological changes mainly refer to interaction of vascular endothelial cell damage, inflammatory response, proliferation of smooth muscle cells and the change of the matrix [3, 4]. Coronary artery disease contains a wide range of clinical manifestations, including subclinical asymptomatic atherosclerosis and some clinical complications, such as angina, myocardial infarction and sudden cardiac of death [5-7]. At present, CAD is considered to be one of the most important fatal diseases in industrial countries and has become one of the world's leading causes of death and disability [8]. More than 80% of patients occurred in low and middle income countries [9]. The incidence of CAD in western developed countries was also high, studies showed that CAD accounted for the first rank deaths in cardiovascular disease in the United States and Europe and other countries [10, 11]. Chinese current incidence of this disease is also increasing year by year, leading to the economic and social a heavy burden [12, 13].

Several studies have shown that atherosclerotic lesions are inflammatory processes in the blood vessels in different phase, with endothelial dysfunction, fat formation, and a series of concurrent diseases [14]. The typical cell types of atherosclerosis are full of lipid laden macrophages [15]. These macrophages are induced to vascular wall by chemokine, gathering and causing inflammation in local area and expressing and releasing of inflammatory mediators [16]. The primary inflammatory cells in the process of atherosclerosis are mainly CD4+ T

Table 1. PCR primers used in this study

SNPs	Primers	Sequences (5'-3')
rs2243250 (C/T)	Forward	AAACTAGGCCTCACCTGATACG
	Reverse	TGCATAGAGGCAGAATAACAGG
rs2227284 (T/C)	Forward	GAGGTGAGACCCATTAATAG
	Reverse	CGTGGATTGCTTAGCTTCCT
Rs2070874 (T/C)	Forward	ACGTTGGATGTGCATCGTTAGCTTCTCCTG
	Reverse	ACGTTGGATGGAGGTGAGACCCATTAATAG
rs1801275 (Q/R)	Forward	GAGGAAGTAGAACCCGAGATGC
	Reverse	GCAGCCAGGAATGAGGTCTT

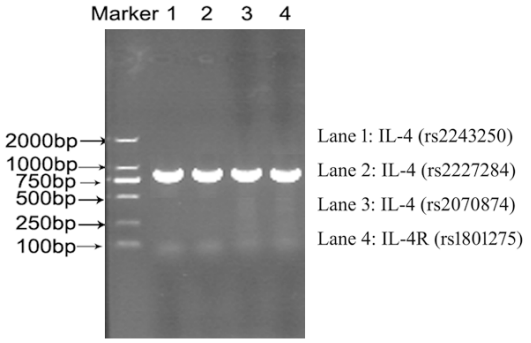

Figure 1. PCR amplification electrophoresis for IL-4 gene.

cells, releasing a series of proinflammatory cytokines for Th1. These cytokines, such as IFN-γ and TNF-α, not only induce the expression of chemokines, but also activate the cell activity of vascular wall [17]. Recent studies found that Th2 cells played an important role in the development of atherosclerosis [18, 19]. Interleukin 4 (IL-4) was discovered in 1982, with a variety of biological functions of cytokines, and it is mainly secreted by activated T cells and mononuclear cells [20]. The mast cells and basophilic granule cells also synthesize and secrete this cytokine [20]. IL-4 plays an important role in regulating the differentiation and activation of T and B lymphocytes and promoting the immune response to Th2 cells [21]. IL-4 is the characteristic cytokine of Th2 cells, which has the inhibition effect on the inflammation and transplant rejection caused by the cytokine network to the Th1, and has a strong and broad biological activity [22]. Some researchers showed that the risk of CAD was related to IL-6 [23], IL-10 [24] and IL-17 [25] expression and genetic polymorphisms. However, still no correlation of IL-4 and its gene polymorphisms with CAD risk has been reported. Therefore, we firstly performed this analysis of the relations between IL-4 genetic polymorphisms (rs2243250, rs2227284, rs2070874 and rs1801275) and CAD risk, preliminarily made clearly the role for IL-4 genetic polymorphisms in the pathogenesis of coronary artery disease.

Materials and methods

Subjects

A total of 420 cases of coronary artery disease patients were collected from June 2010 to October 2015 in the department of Geriatrics of the First Hospital of Shijiazhuang City. Coronary angiography was performed and all the patients were positive (at least one branch coronary artery diameter stenosis ≥50%). All the subjects were also enrolled according to the WHO criteria for diagnosis of patients with CAD in 1979. There were 281 males and 139 females, and these patients were aged at 37 to 83 years old (60.2±13.3 years).

During the same time period, a total of 420 subjects were enrolled as the control group. All the control subjects received routine physical examination in section for outpatients, except for coronary artery disease. There were 253 male and 167 female, and they aged 35 to 84 years old (58.9±14.3 years). All the subjects were unrelated Chinese Han population; and those with the systemic inflammation, rheumatic disease, tumor, liver and kidney related diseases were excluded. Coronary artery disease group and the control group were matched by age and sex. This study was approved by the ethics committee of The First Hospital of Shijiazhuang City.

Height and weight of all the subjects were collected from medical records. The body mass index (BMI) was calculated from weight and height [BMI=weight/height^2 (kg/cm^2)]. The medical history and the lifestyles were obtained through the questionnaire survey method.

Five ml peripheral venous blood were collected from each subjects, and the blood samples were separated serum and stored at -80°C for lipid analysis; the white cell was handled with

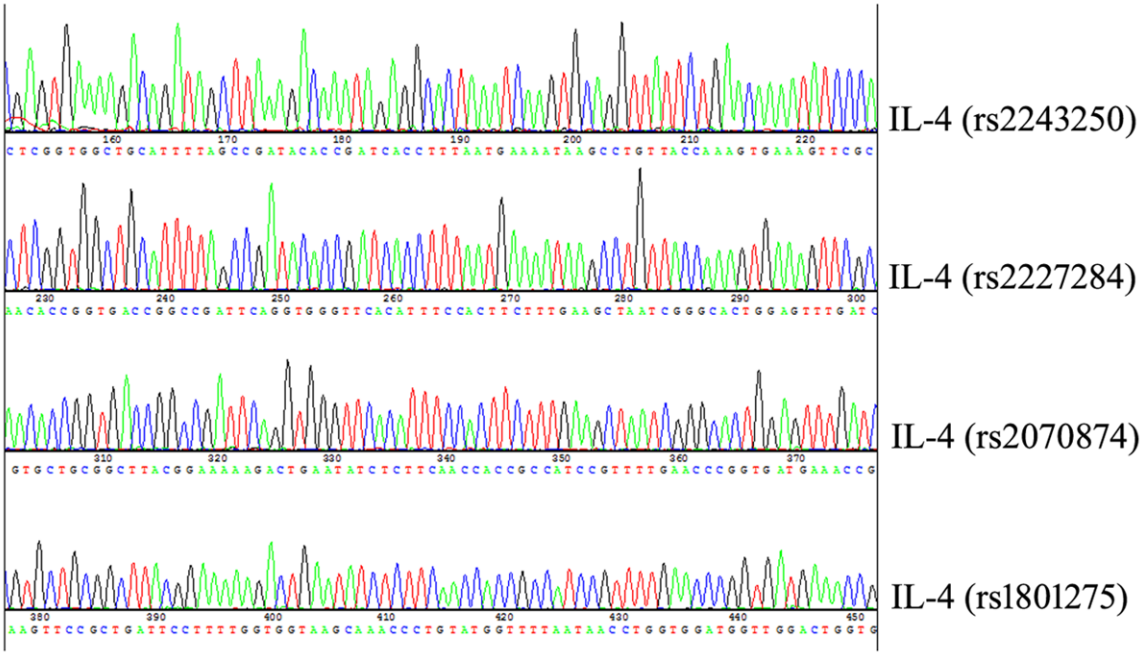

Figure 2. DNA sequence results of IL-4 rs2243250, rs2227284, rs2070874 and rs1801275.

Blood DNA extraction kit to extract genomic DNA and stored at -20°C preservation for genotype analysis.

Lipid measurement

Total cholesterol (TC) was detected by cholesterol oxidase-Oxidase-4-amino antipyrine and phenol method (CHOD-PAP); Triglyceride (TG) was determined by lipid lipoprotein lipase-glycerol phosphate oxidase-Oxidase 4 amino antipyrine and phenol method (GPO-PAP). High density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were verified by using uniform phase method. Lipoprotein (apo) AI and apoB were detected by immune transmission turbidimetry (ITA). All of the above methods were carried out on the AU1000/2700 automatic biochemical analyzer (OLYMPUS).

DNA extraction and genotyping

According to the manufacturer's instructions, genomic DNA was extracted by QIAamp DNA Blood Mini DNA extraction kit. PCR amplification and DNA sequence analysis method were used. The primers were designed with Autoprimer tool (http://www.autoprimer.com), and synthesized by the Shanghai SangGong production company, the primer sequences of rs2243250, rs2227284, rs2070874 and rs1801275 were shown in **Table 1**. PCR reaction system was 50 μl including: dNTPs 75 μM, 20 ng genomic DNA, 50 nM primers, $MgCl_2$ 3.5 mM and Hotstar Taq 0.5 U enzyme. The 96 hole plate was used for PCR reaction: 95°C 15 min, 94°C 30 s, 55°C 30 s, 72°C 60 s, 40 cycles, and the last was 72°C 7 min, finally placed at 4°C. The PCR amplification instrument was ABI PCR amplification (ABI, USA). The PCR products of rs2243250, rs2227284, rs2070874 and rs1801275 were sequenced by the business company (TaKaRa); the results were analyzed by DNAStar software, and compared by MEGA5.0 software (**Figures 1** and **2**).

Statistical analysis

SPSS Statistics for Windows, Version 17.0. (Chicago: SPSS Inc.) were used for statistical analysis. The general data of the patients group and the control group were compared by t test or χ^2 test. The genotype frequency of the control population was tested by χ^2 goodness-of-fit Hardy-Weinberg equilibrium (HWE), in order to describe the population representation. The differences of genotype frequencies, allele frequencies and haplotype frequencies between patients and controls were tested by χ^2 test,

Table 2. The general situation comparison between patients and control groups

Groups	CAD patients	%	Control	%	t test or χ^2	*P* value
Age						
<60	224	53.33	227	54.05		
≥60	196	46.67	193	45.95	0.05	0.82
Gender						
Female	178	42.38	238	56.67		
Male	242	57.62	182	43.33	17.54	<0.001
Hypertension						
No	293	69.76	349	83.10		
Yes	127	30.24	71	16.90	39.42	<0.001
Type 2 diabetes mellitus						
No	296	70.48	363	86.43		
Yes	124	29.52	57	13.57	19.41	<0.001
BMI≥30						
No	271	64.52	312	74.29		
Yes	149	35.48	108	25.71	32.66	<0.001
Smoking						
No	234	55.71	260	61.90		
Yes	186	44.29	160	38.10	87.04	<0.001
Alcohol drinking						
No	197	46.90	243	57.86		
Yes	223	53.10	177	42.14	1.68	0.17
TC (mg/dL)	197.5±36.9		171.4±31.8		9.81	<0.001
TG (mg/dL)	133.4±43.3		116.7±31.4		5.74	<0.001
LDL-c (mg/dL)	110.5±27.9		96.7±8.9		8.46	<0.001
HDL-c (mg/dL)	37.6±8.8		43.7±5.9		10.1	<0.001

and the single factor and multiple factors logistic regression were used to calculate the OR values and 95% CI values. Interaction between genotype and environment related factors (BMI, hypertension, alcohol drinking and other factors) were analyzed by the logistic regression analysis. P<0.05 was recognized as statistically significant.

Results

The general situation of the patients and control group were shown in **Table 2**. The gender, hypertension, type 2 diabetes mellitus, obesity and smoking were statistical differences between the two groups (P<0.05), while the ages, alcohol drinking had no significant differences between patients and control groups (P>0.05). The mean values of TC, TG, LDL-c and HDL-c also had statistical significant differences between two groups (P<0.05).

Distributions of rs2243250 and rs1801275 loci in the control group were in accordance with Hardy Weinberg equilibrium (χ^2= 0.08 and 0.24), while rs2227284 and rs2070874 were not. The distributions and allele frequencies of the four loci between the patients and control groups were shown in **Table 3**. Alleles and genotypes of IL-4 rs2227284, rs2070874 and IL-4R rs1801275 between the CAD patients and control group had no statistical significances (P>0.05), while the IL-4 rs2243250 displayed significant differences between CAD patients and the control groups (P<0.05). After adjustment for age, smoking, drinking and family history of CAD, multivariate logistic regression analysis showed that the CT and TT genotypes of IL-4 rs2243250 were associated with an increased risk of coronary artery disease when compared with the CC genotype (adjusted OR=1.44, 95% CI: 1.05-1.89; 2.08, 95% CI: 1.41-3.31).

For further analysis, we performed a gene-environment interaction analysis for the susceptibility to CAD (**Table 4**). Compared with the CC genotype, patients carrying the CT genotype of rs2243250 had interaction with smoking showed a higher coronary artery disease prevalence risk (OR=2.14, 95% CI: 1.37-3.62), while other factors and TT genotypes had no statistical significance with environmental factors.

Discussion

With the development of the aging population, the incidence of atherosclerosis disease is gradually increasing [26]. Economic development and urbanization have promoted the changes of human dietary and lifestyle habits, including excessive intake of saturated fatty acids, lack of exercise, etc, which led to the occurrence of atherosclerosis [27].

It is recognized that the process of inflammation is the most important factor in the process

Table 3. Distribution and frequency of four loci with susceptibility of CAD in this study

Gene polymorphisms		CAD patients	%	Controls	%	HWE	OR (95% CI)[1]	*P* value
rs2243250 (589 C/T)	CC	171	40.71	219	52.14		1.0 (Ref.)	-
	CT	161	38.33	147	35		1.44 (1.05-1.89)	0.02
	TT	88	20.96	54	12.86	0.08	2.08 (1.41-3.31)	0.03
Allele	C	503	59.88	585	69.64		1.0 (Ref.)	-
	T	337	40.12	255	30.36		1.52 (1.26-1.92)	<0.001
rs2227284 (107 T/C)	TT	270	64.29	289	68.81		1.0 (Ref.)	-
	TC	93	22.14	86	20.48		1.15 (0.81-1.64)	0.34
	CC	57	13.57	45	10.71	<0.05	1.34 (0.86-2.17)	0.17
Allele	T	633	75.36	664	79.05		1.0 (Ref.)	-
	C	207	24.64	176	20.95		1.22 (0.91-1.55)	0.07
rs2070874 (33 T/C)	TT	122	29.05	138	32.86		1.0 (Ref.)	-
	TC	181	43.1	174	41.43		1.15 (0.83-1.61)	0.29
	CC	117	27.85	108	25.71	<0.05	1.21 (0.79-1.76)	0.24
Allele	T	425	50.59	450	53.57		1.0 (Ref.)	-
	C	415	49.41	390	46.43		1.11 (0.89-1.31)	0.21
rs1801275 (576 Q/R)	QQ	157	37.38	170	40.48		1.0 (Ref.)	-
	QR	180	42.86	176	41.9		1.07 (0.79-1.44)	0.51
	RR	83	19.76	74	17.62	0.24	1.21 (0.77-1.81)	0.36
Allele	Q	494	58.81	516	61.43		1.0 (Ref.)	-
	R	346	41.19	324	38.57		1.04 (0.88-1.32)	0.25

[1]Adjusted for age, gender, medical history, alcohol drinking and smoking.

of atherosclerosis. In addition, some subclinical inflammatory states, such as diabetes and rheumatoid arthritis, are also considered to be a strong risk factor for cardiovascular disease [11]. The etiology of CAD has not yet been fully understood; studies showed that the incidence of coronary artery disease was mainly caused by interaction between environmental, genetic and other related factors [28].

The pathogenesis of coronary artery disease is complex and involves many risk factors. Traditional risk factors, such as: smoking, hypertension, diabetes, high blood lipids, has been studied clearly. In our study, we showed that the gender, hypertension, type 2 diabetes mellitus, obesity and smoking were statistical different between CAD patient and control; the values of TC, TG, LDL-c and HDL-c also had statistical significant difference. These finding were similar with previous researches [9, 10, 12, 13, 29-32].

In recent years, studies indicated that not all individuals with similar risk factors of CAD did not all occur CAD, suggesting that some genetic factors contribute to the risk of coronary artery disease. Yang et al. [33] conducted a case-control study to investigate the genetic variants Interleukin-1β, IL-6, IL-8 and IL-10 in the development of coronary artery disease. Their results showed that the frequencies of the CC genotype and the C allele of IL-6 (174 G/C) were significantly correlated with a higher risk of CAD. In addition, the AG and GG genotypes and the G allele of IL-10 (1082 A/G) were also significantly associated with a high risk of CAD. However, IL-1β (3953 C/T), IL-8 (251 T/A), and IL-10 (819 C/T) did not significantly correlate with CAD risk. Zheng et al. [34] found that the CC and TC+CC genotypes of rs3748067 of IL-17A were connected with increased risk of CAD in comparison to the wide-type genotype, particularly in smokers, and the ORs (95% CIs) were 3.81 (2.11-7.16), 1.54 (1.11-2.14), respectively. Therefore, the development of CAD had closely relations with IL family and its polymorphisms.

IL-4, as a characteristic cytokine of Th2 cells, has a role in promoting the occurrence and development of Th2 in the inflammatory response, and there are many ways to promote the development of the inflammatory response [35]. The biological effect of IL-4 is mediated by binding its receptor. IL-4 receptor (IL4R) is wide-

Table 4. Gene-environmental factors interaction analysis of rs2243250 genotype with susceptibility to CAD

Groups	CC		CT		OR value	*P* value	TT		OR value	*P* value
	Patients	Control	Patients	Control			Patients	Control		
BMI (kg/m^2)										
<30	115	162	101	110	1.39 (0.87-2.04)	0.07	55	40	1.21 (0.80-1.54)	0.06
≥30	56	57	60	37	1.74 (0.91-3.32)	0.08	33	14	1.42 (0.81-3.02)	0.06
Hypertension										
No	123	179	110	124	1.33 (0.81-2.10)	0.07	60	46	1.23 (0.79-2.01)	0.06
Yes	48	40	51	23	1.83 (0.86-3.99)	0.09	28	8	1.81 (0.82-3.45)	0.06
Type 2 diabetes mellitus										
No	123	186	112	130	1.41 (0.86-1.97)	0.06	61	47	1.31 (0.76-1.90)	0.06
Yes	48	33	49	17	2.07 (0.90-4.88)	0.07	27	7	2.00 (0.88-3.87)	0.06
Alcohol consumption										
No	79	125	76	86	1.45 (0.89-2.42)	0.06	42	32	1.25 (0.82-2.02)	0.06
Yes	92	94	85	61	1.53 (0.95-2.52)	0.07	46	22	1.43 (0.94-2.72)	0.06
Smoking										
No	99	137	92	95	1.07 (0.70-1.69)	0.67	43	28	1.01 (0.74-1.61)	0.67
Yes	72	82	69	52	2.14 (1.37-3.62)	0.003	45	26	1.14 (0.77-2.62)	0.09

ly distributed in endothelial, synovial, hematopoietic, muscle cells and brain tissue, and some tumor cells could also express IL-4R. Numerous studies have investigated the association between IL-4 or IL-4R polymorphisms and risk of cancers or their mortality [36], but never referred to the risk of coronary artery disease. Therefore, we firstly investigated the association between IL-4 polymorphisms and risk of CAD. In our study, IL-4 rs2243250 has statistical significant difference between two groups, CT and TT genotype and T allele frequencies were significantly higher than those in the control group, indicated that these genotypes or allele could increase the risk of CAD. In another side, we still found that the CT genotypes with smoking related environment have positive interaction, increasing the risk of CAD. Previous work mainly focused on other cytokines [34], and they also found smoking had relations with gene SNPs, however, for IL-4, we firstly reported such relationships.

The results of this study have two limitations. First, the patients were selected from a single region in China; therefore, the sample may not be representative of the general population. However, the genotype frequencies of the IL-4 and IL-4R polymorphisms were in accordance with the HWE, indicating that the samples could be representative of the general population. Second, the sample size was quite small, resulting in low statistical power to compare the differences between groups.

Our results show IL-4 rs2243250 (589 C/T) allele is associated with coronary artery disease risk in Chinese population, and CT and TT genotypes of IL-4 rs2243250 have a higher risk of developing CAD as compared with the CC genotype; the CT genotypes of IL-4 rs2243250 had interaction with tobacco smoking in the risk of CAD. Further large-scale studies should be conducted to gain better insight into the impact of IL-4 and L-4R sequence variations on CAD risk.

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Disclosure of conflict of interest

None.

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References

[1] Gudbjartsson T, Andersen K, Danielsen R, Geirsson A, Thorgeirsson G. [Review on coronary artery disease-part I: epidemiology,

pathophysiology, clinical presentation and work-up]. Laeknabladid 2014; 100: 667-676.
[2] Zhang JM, Zhong L, Su B, Wan M, Yap JS, Tham JP, Chua LP, Ghista DN, Tan RS. Perspective on CFD studies of coronary artery disease lesions and hemodynamics: a review. Int J Numer Method Biomed Eng 2014; 30: 659-680.
[3] James TN. Clinico-pathological correlations in coronary artery disease. Med J Aust 1972; 2 Suppl: 10-12.
[4] Yahagi K, Davis HR, Arbustini E, Virmani R. Sex differences in coronary artery disease: pathological observations. Atherosclerosis 2015; 239: 260-267.
[5] Urowitz MB, Gladman D, Ibanez D, Fortin P, Sanchez-Guerrero J, Bae S, Clarke A, Bernatsky S, Gordon C, Hanly J, Wallace D, Isenberg D, Ginzler E, Merrill J, Alarcon G, Steinsson K, Petri M, Dooley MA, Bruce I, Manzi S, Khamashta M, Ramsey-Goldman R, Zoma A, Sturfelt G, Nived O, Maddison P, Font J, van Vollenhoven R, Aranow C, Kalunian K, Stoll T, Buyon J. Clinical manifestations and coronary artery disease risk factors at diagnosis of systemic lupus erythematosus: data from an international inception cohort. Lupus 2007; 16: 731-735.
[6] Tresch DD, Aronow WS. Clinical manifestations and diagnosis of coronary artery disease. Clin Geriatr Med 1996; 12: 89-100.
[7] Kuznetsov VA, Yaroslavskaya EI, Bessonova MI, Bessonov IS, Zyrianov IP, Kolunin GV, Krinochkin DV, Maryinskih LV, Bektasheva AS. Clinical manifestations and risk factors of coronary artery disease in patients with diabetes mellitus in western Siberia. Int J Circumpolar Health 2010; 69: 278-284.
[8] Brown RA, Shantsila E, Varma C, Lip GY. Epidemiology and pathogenesis of diffuse obstructive coronary artery disease: the role of arterial stiffness, shear stress, monocyte subsets and circulating microparticles. Ann Med 2016; 48: 444-455.
[9] Mack M, Gopal A. Epidemiology, traditional and novel risk factors in coronary artery disease. Heart Fail Clin 2016; 12: 1-10.
[10] Puymirat E. [Epidemiology of coronary artery disease]. Rev Prat 2015; 65: 317-320.
[11] Mack M, Gopal A. Epidemiology, traditional and novel risk factors in coronary artery disease. Cardiol Clin 2014; 32: 323-332.
[12] Hata J, Kiyohara Y. Epidemiology of stroke and coronary artery disease in Asia. Circ J 2013; 77: 1923-1932.
[13] Bi YF, Mao JY, Wang XL, Hou YZ, Lu YZ, Soh SB, Zhang BL. Clinical epidemiology survey of the traditional Chinese medicine etiology and syndrome differentiation of coronary artery disease: study protocol of a multicenter trial. Zhong Xi Yi Jie He Xue Bao 2012; 10: 619-627.
[14] Moga A, Haragus S, Vlaicu R, Dobosi T, Opincaru A. [The role of inflammatory and atherosclerotic processes in certain arterial diseases]. Probl Cardiol 1959; 4: 63-70.
[15] Zhang R, Ma J, Xia M, Zhu H, Ling W. Mild hyperhomocysteinemia induced by feeding rats diets rich in methionine or deficient in folate promotes early atherosclerotic inflammatory processes. J Nutr 2004; 134: 825-830.
[16] Ahmed MA, Elosaily GM. Role of oxytocin in deceleration of early atherosclerotic inflammatory processes in adult male rats. Int J Clin Exp Med 2011; 4: 169-178.
[17] Kalaiyarasu S, Bhatia S, Mishra N, Sood R, Kumar M, SenthilKumar D, Bhat S, Dass Prakash M. Elevated level of pro inflammatory cytokine and chemokine expression in chicken bone marrow and monocyte derived dendritic cells following LPS induced maturation. Cytokine 2016; 85: 140-147.
[18] Meng K, Zeng Q, Lu Q, Lin Y, Wu B, Yu K, Dong Z, Zhang J, Chai M, Liu Y, Ji Q, Zhou Y. Valsartan attenuates atherosclerosis via upregulating the Th2 immune response in prolonged angiotensin II-treated apoe(-/-) mice. Mol Med 2015; 21: 143-153.
[19] Vinson A, Curran JE, Johnson MP, Dyer TD, Moses EK, Blangero J, Cox LA, Rogers J, Havill LM, Vandeberg JL, Mahaney MC. Genetical genomics of Th1 and Th2 immune response in a baboon model of atherosclerosis risk factors. Atherosclerosis 2011; 217: 387-394.
[20] Tay SS, Plain KM, Bishop GA. Role of IL-4 and Th2 responses in allograft rejection and tolerance. Curr Opin Organ Transplant 2009; 14: 16-22.
[21] Stott B, Lavender P, Lehmann S, Pennino D, Durham S, Schmidt-Weber CB. Human IL-31 is induced by IL-4 and promotes TH2-driven inflammation. J Allergy Clin Immunol 2013; 132: 446-454 e445.
[22] Kim S, Karasuyama H, Lopez AF, Ouyang W, Li X, Le Gros G, Min B. IL-4 derived from Non-T cells induces basophil- and IL-3-independent Th2 immune responses. Immune Netw 2013; 13: 249-256.
[23] Lim GB. Coronary artery disease: IL-6 signaling linked with CHD. Nat Rev Cardiol 2012; 9: 313.
[24] Weng KP, Hsieh KS, Hwang YT, Huang SH, Lai TJ, Yuh YS, Hou YY, Lin CC, Huang SC, Chang CK, Lin MW, Ger LP. IL-10 polymorphisms are associated with coronary artery lesions in acute stage of Kawasaki disease. Circ J 2010; 74: 983-989.
[25] Csiszar A, Ungvari Z. Synergistic effects of vascular IL-17 and TNFalpha may promote coronary artery disease. Med Hypotheses 2004; 63: 696-698.

[26] Pearson TA. Coronary arteriography in the study of the epidemiology of coronary artery disease. Epidemiol Rev 1984; 6: 140-166.
[27] Weber T, Lang I, Zweiker R, Horn S, Wenzel RR, Watschinger B, Slany J, Eber B, Roithinger FX, Metzler B. Hypertension and coronary artery disease: epidemiology, physiology, effects of treatment, and recommendations: a joint scientific statement from the Austrian Society of Cardiology and the Austrian Society of Hypertension. Wien Klin Wochenschr 2016; 128: 467-479.
[28] Yasuda S. [Data review of coronary artery disease and its risk factors; Insights from epidemiology and registry study and nationwide survey]. Nihon Rinsho 2016; 74 Suppl 4 Pt 1: 13-18.
[29] Beadenkopf WG, Daoud A, Marks RU, Kinch SH. Epidemiology and pathology of coronary artery disease. I. Method of study. J Chronic Dis 1960; 12: 504-520.
[30] Borhani NO. Forum on cardiovascular disease epidemiology. Introduction: opportunities for primary prevention of coronary artery disease. Prev Med 1975; 4: 482-490.
[31] Ferrieres J. [The epidemiology of coronary artery disease in older than 75 years]. Arch Mal Coeur Vaiss 2006; 99 Spec No 4: 7-12.
[32] Haffner SM. Epidemiology of insulin resistance and its relation to coronary artery disease. Am J Cardiol 1999; 84: 11J-14J.
[33] Yang HT, Wang SL, Yan LJ, Qian P, Duan HY. Association of interleukin gene polymorphisms with the risk of coronary artery disease. Genet Mol Res 2015; 14: 12489-12496.
[34] Zheng XS, Wang S, Ni M. Association between interleukin 17A gene polymorphisms and risk of coronary artery disease. Genet Mol Res 2016; 15.
[35] Volpin G, Cohen M, Assaf M, Meir T, Katz R, Pollack S. Cytokine levels (IL-4, IL-6, IL-8 and TGFbeta) as potential biomarkers of systemic inflammatory response in trauma patients. Int Orthop 2014; 38: 1303-1309.
[36] Zhenzhen L, Xianghua L, Qingwei W, Zhan G, Ning S. Three common polymorphisms in the IL-4 gene and cancer risk: a meta-analysis involving 5,392 cases and 6,930 controls. Tumour Biol 2013; 34: 2215-2224.