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

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Genet. Mol. Res. 15 (1): gmr.15017074
Received October 24, 2015
Accepted December 5, 2015
Published February 5, 2016
DOI http://dx.doi.org/10.4238/gmr.15017074

ABSTRACT. Coronary artery disease (CAD) represents a leading cause of morbidity and mortality worldwide, and genetic factors contribute to the development of this disease. We conducted a case-control study to assess the association between interleukin 17A (*IL17A*) rs2275913 and rs3748067 polymorphisms and development of CAD. A total of 372 CAD patients and 372 healthy controls were recruited in our investigation between January 2013 and December 2014. Genotyping of *IL17A* rs2275913 and rs3748067 was carried out using polymerase chain reaction combined with restriction fragment length polymorphism. Logistic regression analysis revealed that CC [odds ratio (OR) = 3.81, 95% confidence interval (CI) = 2.11-7.16] and TC+CC (OR = 1.54, 95%CI = 1.11-2.14) rs3748067 genotypes were associated with an increased risk of CAD compared to the TT variant. Individuals carrying the TC+CC genotype were more likely to have a higher risk of CAD if they were smokers, with an adjusted OR (and 95%CI) of 2.20 (1.31-3.71). In conclusion, we suggest that the CC and TC+CC genotypes

of rs3748067 are connected with increased risk of CAD in comparison to the wide-type genotype, particularly in smokers.

Key words: Interleukin 17; Polymorphism; Coronary artery disease

INTRODUCTION

Coronary artery disease (CAD) represents a leading cause of death worldwide, and more than 80% of cases occur in low-to-middle income countries (Greenland et al., 2010). It is well known that the development of CAD is a complex, multistep, and multifactorial process, involving various environmental factors. Previous studies have reported that the male gender, hypertension, tobacco use, alcohol consumption, and diabetes mellitus as well as high serum cholesterol contribute to the development of CAD (Greenland et al., 2010). However, not all individuals exposed to similar risk factors of CAD influences go on to develop this disease, suggesting that hereditary factors also contribute to the susceptibility to this condition.

Previous study has reported that the inflammation plays an important role in the risk of atherosclerosis (Weber and Hristov, 2015). The comparatively new interleukin 17 (IL17) cytokine family consists of six members (IL17A to F), which has been implicated in many chronic inflammatory diseases, such as ulcerative colitis risk, asthma and tuberculosis (Li et al., 2014; Bulat-Kardum et al., 2015; Narbutt et al., 2015; Isailovic et al., 2015). The human *IL17A* gene, located in 6q12, is the most important member of the IL17 group. Previous studies have tested the association between *IL17* gene polymorphisms and risk of cardiovascular disease (Pei et al., 2009; Zhang et al., 2011; Vargas-Alarcón et al., 2015), but with inconclusive results. Therefore, we performed a case-control study to evaluate the association between two common *IL17A* SNPs, rs2275913 and rs3748067, and development of CAD.

MATERIAL AND METHODS

Subjects

A total of 372 CAD patients were consecutively collected from Nanyang City Center Hospital between January 2013 and December 2014. Individuals were diagnosed using angiography, with CAD being defined as a stenosis diameter of 50% in any of the main coronary arteries. Patients with congenital heart disease, autoimmune-related disease, malignant tumor, or serious renal or liver diseases were excluded from this study.

In total, 372 health control subjects attending regular health checkups at our hospital over the same period were also recruited. Individuals were confirmed as having no history of CAD, autoimmune-related disease, or renal disease. Each control subject was matched to each case by gender and age.

Demographic and lifestyle data of CAD patients and control subjects were collected using a structured questionnaire, and included details regarding age, gender, body mass index, hypertension, alcohol consumption, and tobacco smoking. Clinical data collected from medical records consisted of total cholesterol (TC), low- and high-density lipoprotein cholesterol (LDL-C and HDL-C, respectively), and triglyceride (TG) measurements.

A signed informed consent form was obtained from each subject before participation. The

collection of blood samples for this study was approved in advance by the Ethics Committee of Nanyang City Center Hospital.

Genetic analysis

Each participant was asked to provide a 5-mL peripheral venous blood sample after enrolling in our study, from which DNA was then isolated by salt extraction. Genotyping of *IL17A* rs2275913 and rs3748067 was performed by polymerase chain reaction (PCR) combined with restriction fragment length polymorphism. The primers for *IL17A* rs2275913 and rs3748067 were designed using the MassARRAY Assay Design 3.1 software (Sequenom, San Diego, CA, USA). The resulting PCR fragments were subsequently digested with restriction enzymes specific to the sequence of interest, namely *Bst*ENI for rs3748067 and *Ava*II for rs2275913. Amplification conditions were as follows: an initial DNA denaturation step at 94°C for 5 min, then 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min, followed by a final extension step at 72°C for 5 min.

Statistical analysis

Differences in demographic and clinical characteristics between CAD patient and control groups were compared using the Pearson chi-square test or the Student *t*-test. Genotype distributions in the control group were tested for deviation from Hardy-Weinberg equilibrium. Pearson chi-square test was used to examine differences in genotypic distribution between CAD patients and controls. Odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated using multiple-logistic regression models adjusted for confounding factors. Statistical analyses were performed using the SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), and all tests were two-sided, with P values <0.05 indicating statistical significance.

RESULTS

The demographic and clinical data of CAD patients and control subjects are presented in Table 1. The chi-square test revealed that individuals with CAD were more likely to have a higher BMI, suffer from hypertension, smoke tobacco, and drink alcohol ($P < 0.05$). Moreover, CAD patients demonstrated higher TC, LDL-C, and TG levels, and lower HDL-C when compared with control subjects ($P < 0.05$).

In the control group, the distribution of rs2275913 genotypes was consistent with Hardy-Weinberg equilibrium ($P = 0.40$), but that of rs3748067 was not ($P = 0.002$). Using the chi-square test, a significant difference in the frequencies of rs3748067 genotypes was established between the CAD and control groups (chi-square = 23.96, $P < 0.001$). Logistic regression analysis showed that the CC and TC+CC rs3748067 genotypes, with adjusted ORs (and 95%CIs) of 3.81 (2.11-7.16) and 1.54 (1.11-2.14), respectively, were associated with an increased risk of CAD when compared with the TT genotype (Table 2). However, no significant relationship between rs2275913 and CAD was found.

We also conducted gene-environment interaction analysis to investigate the influence of rs3748067, in combination with clinical and demographic characteristics, on CAD risk. We found that individuals carrying the TC+CC genotype were more likely to have CAD if they were smokers (adjusted OR = 2.20, 95%CI = 1.31-3.71; Table 3). However, no significant interaction between this polymorphism and BMI, hypertension, diabetes mellitus, or alcohol consumption was seen to effect CAD risk ($P > 0.05$).

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

	CAD cases (N = 372)	%	Controls (N = 372)	%	Chi-square or *t*- test	P value
Mean age (years)	62.15 ± 11.30		61.74 ± 10.95		0.50	0.31
<60	166	44.62	172	46.24		
≥60	206	55.38	200	53.76	0.20	0.66
Gender						
Male	280	75.27	280	75.27		
Female	92	24.73	92	24.73	0.00	1.00
BMI (kg/m^2)						
<24	244	65.59	276	74.19		
≥24	128	34.41	96	25.81	6.54	0.01
Hypertension						
No	262	70.43	307	82.53		
Yes	110	29.57	65	17.47	15.13	< 0.001
Alcohol consumption						
Non-drinkers	173	46.51	213	57.26		
Drinkers	199	53.49	159	42.74	8.61	0.003
Tobacco smoking						
Non-smokers	196	52.69	235	63.17		
Smokers	176	47.31	137	36.83	8.39	0.004
TC (mmol/dL)	197.30 ± 51.08		172.40 ± 30.52		7.45	< 0.001
LDL-C (mmol/dL)	112.65 ± 27.42		98.30 ± 26.45		6.71	< 0.001
HDL-C (mmol/dL)	36.60 ± 8.24		42.65 ± 7.60		9.61	< 0.001
TGs (mmol/dL)	135.35 ± 42.10		114.30 ± 28.55		7.37	< 0.001

CAD = coronary artery disease; BMI = body mass index; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides.

Table 2. *IL17A* rs2275913 and rs3748067 genotype distributions and their association with coronary artery disease risk.

SNP	Base change	Patients	%	Controls	%	P value for HWE	OR (95%CI)[1]	P value
rs2275913								
GG		162	43.55	179	48.12		1.0 (Ref.)	-
GA		170	45.7	163	43.82		1.15 (0.84-1.58)	0.36
AA	G>A	40	10.75	30	8.06	0.4	1.47 (0.85-2.57)	0.14
GA+AA		210	56.45	193	51.88		1.20 (0.89-1.62)	0.21
rs3748067								
TT		243	65.32	276	74.19		1.0 (Ref.)	-
TC		73	19.62	79	21.24		1.05 (0.72-1.53)	0.79
CC	C>T	57	15.32	17	4.57	0.002	3.81 (2.11-7.16)	< 0.001
TC+CC		130	34.95	96	25.81		1.54 (1.11-2.14)	0.007

[1]Adjusted for gender, age, body mass index, hypertension, diabetes mellitus, alcohol consumption, tobacco smoking, total cholesterol, low- and high-density lipoprotein cholesterol, and triglyceride measurements. SNP = single nucleotide polymorphism; HWE = Hardy-Weinberg equilibrium; OR = odds ratio; CI = confidence interval; Ref. = reference.

Table 3. Interaction between the rs3748067 polymorphism and demographic and clinical characteristics in risk of coronary artery disease.

Variable	rs3748067				OR (95%CI)	P value
	TT		TC+CC			
	Cases	Controls	Cases	Controls		
BMI (kg/m^2)						
<24	163	204	81	72	1.41 (0.95-2.09)	0.08
≥24	80	72	48	24	1.80 (0.97-3.39)	0.06
Hypertension						
No	174	226	88	81	1.41 (0.87-2.06)	0.06
Yes	69	50	41	15	1.98 (0.95-4.28)	0.06
Diabetes mellitus						
No	175	235	90	85	1.42 (0.98-2.06)	0.05
Yes	68	41	39	11	2.14 (0.94-5.13)	0.05
Alcohol consumption						
Non-drinkers	112	157	61	56	1.53 (0.96-2.42)	0.06
Drinkers	131	119	68	40	1.54 (0.95-2.53)	0.07
Tobacco smoking						
Non-smokers	141	173	55	62	1.09 (0.69-1.70)	0.7
Smokers	102	103	74	34	2.20 (1.31-3.71)	0.002

OR = odds ratio; CI = confidence interval; BMI = body mass index

DISCUSSION

Genetic susceptibility to cardiovascular disease has gained an increasing amount of attention as a research theme, leading to investigations of polymorphisms involved in the development of CAD. Inflammation and related cytokines are involved in both innate and acquired immune responses, and play a critical role in the inflammatory response, which contributes to the susceptibility to cardiovascular disease.

Recently, many studies have assessed the association between *IL17A* and susceptibility to many cardiovascular diseases (Zeng et al., 2013; Erbel et al., 2014; Lifanov et al., 2014; Vargas-Alarcón et al., 2015; Mormile, 2015; Wang et al., 2015). Based on an experimental investigation, Vargas-Alarcón et al. (2015) reported that the *IL17A* gene is involved in inflammatory immune responses and neuronal damage, including premature coronary diseases. In addition, Lifanov et al. (2014) reported an association between the 197A allele of the *IL17A* gene and low myocardial growth in athletes, suggesting that they have a pathogenetic function in this disease. Erbel et al. (2014) reported that functional blockade of *IL17A* prevents atherosclerotic lesion.

Three previous studies have assessed the association between *IL17* gene polymorphisms and cardiovascular diseases. Pei et al. (2009) conducted a case-control examination of a Chinese population, the results of which indicated that the *IL17F* rs763780 polymorphism is unlikely to contribute to the development of myocardial infarction. In another case-control study of a Chinese population, Zhang et al. (2011) reported that *IL17A* rs8193037 is associated with increased risk of CAD and contributes to elevated *IL17A* expression in acute myocardial infarction. Vargas-Alarcón et al. (2015) conducted a case-control study with 900 premature CAD patients and 667 health controls, concluding that *IL17A* haplotypes contributed to the development of CAD. In the present study, we found that the rs3748067 gene polymorphism was associated with an elevated risk of CAD, which is different with the results of previous studies. Such a discrepancy may be caused by differences in the ethnicities of the populations under investigation, study designs, sample sizes, or

simply by chance. Further studies are greatly needed to confirm our findings.

Two limitations should be considered in the present study. First, selection bias could not be avoided due to the hospital-based design, although the gender and age matching between patients and controls may have reduced this bias. Second, the sample size of patients and controls was relatively small, which may have limited the statistical power to find differences between the two groups. Therefore, studies with larger sample sizes are greatly required to confirm our results.

In summary, we found that the rs3748067 gene polymorphism is associated with an increased risk of CAD, especially with regard to smokers. Further genetic studies with large sample sizes are required to confirm the relationship between *IL17* gene polymorphisms and risk of CAD.

Conflicts of interest

The authors declare no conflict of interest.

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