Association between interleukin-17A polymorphism and coronary artery disease susceptibility in the Chinese Han population

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ABSTRACT. Coronary artery disease (CAD) is a major global health problem. In China, the incidence of CAD and the rate of mortality arising from it have increased every year. Interleukin-17A (IL-17A) is a proinflammatory cytokine produced by activated T cells, and it may be involved in the development of CAD. Genetic polymorphisms in functional regions of the *IL17A* gene have a plausible role in modulating the risk of CAD. To evaluate the role of *IL17A* polymorphisms as a risk factor for CAD, we performed a detailed analysis of possible functional single nucleotide polymorphisms (SNPs) in regulatory regions of *IL17A*. This study examined the potential association between CAD and five SNPs (rs8193037, rs8193036, rs3819024, rs2275913, and rs3748067) of the *IL17A* gene. The allelic or genotypic frequencies of the rs8193037 (promoter region) and rs8193036 (promoter region) polymorphisms in CAD were significantly different from those in healthy controls. The CAD subjects had a significantly lower frequency

of the A allele of rs8193037 (P = 0.009, OR = 1.772, 95%CI = 1.146-2.742) and the T allele of rs8193036 (P = 0.010, OR = 1.754, 95%CI = 1.139-2.701). Strong linkage disequilibrium was observed in one block (D' > 0.9). Significantly fewer T-G-G-A haplotypes (P = 0.045) were found in CAD subjects in block 1. These data suggest that *IL17A* gene polymorphisms confer susceptibility to CAD, and support the notion that dysfunction of IL-17A is involved in the pathophysiological process of CAD.

Key words: Coronary artery disease; Interleukin-17A; Single nucleotide polymorphisms; Chinese Han population

INTRODUCTION

Coronary artery disease (CAD) is the world's leading cause of morbidity, mortality, and disability. The prevalence of CAD and the number of deaths resulting from it are predicted to increase rapidly at least until 2030 (Mathers and Loncar, 2006; Lloyd-Jones et al., 2009). Some studies have consistently demonstrated a substantial genetic influence on the development of CAD, with inherited risk estimates in the range of 40-60% (Roberts and Stewart, 2012; Björkegren et al., 2015). Other studies have suggested that single nucleotide polymorphisms (SNPs) in the interleukin-17A gene (*IL17A*) may relate to CAD (Pei et al., 2009; Zhang et al., 2011).

The protein encoded by *IL17A* is a proinflammatory cytokine produced by activated T cells. It regulates the activities of NF-kappaB and mitogen-activated protein kinases, and can stimulate the expression of IL-6 and cyclooxygenase-2 (PTGS2/COX-2), as well as enhance the production of nitric oxide (NO). IL-17A is involved in the attraction and possible activation of macrophages in atherosclerotic lesions, which suggests a systemic induction of IL-17A with a local effect in the vascular wall owing to IL-17A receptor upregulation (Ge et al., 2013). Increased vascular IL-17A levels can create a proinflammatory microenvironment promoting the development of atherosclerotic vascular disease (Csiszar and Ungvari, 2004). IL-17A-driven inflammation may play a role in the promotion of clinical instability in CAD patients, suggesting that the *IL17A* gene is an excellent candidate target for CAD treatment (Hashmi and Zeng, 2006). At present, experimental and clinical findings support the role of *IL17A* in enhancing CAD risk, but information at a population-based genetic level is limited.

Genetic polymorphisms in the functional regions of *IL17A* have a plausible role in modulating the risk of CAD (Shuang et al., 2015). We hypothesized that functional variants in IL-17A might contribute significantly to a predisposition to develop CAD. The SNPs in the promoter region, untranslated regions (UTRs), and exons of *IL17A* were systematically screened. Five SNPs (rs8193037, rs8193036, rs3819024, and rs2275913 in the promoter region, and rs3748067 in the 3'-UTR) with minor allele frequencies (MAF) greater than 0.05 were selected for *IL17A*, based on a review of the published literature (Pei et al., 2009; Shuang et al., 2015) and a search of HapMap and dbSNP databases (Han Chinese population). These SNPs were further analyzed in an association study.

MATERIAL AND METHODS

Subjects

The present study included 219 unrelated patients with CAD [age = 61.4 ± 6.3 years (mean ± SD)]. The patients underwent the following examinations: coronary angiography, electrocardiography (ECG), blood tests, and/or stress tests. The patients were also interviewed.

The inclusion criteria were: 1) at least one diseased vessel (≥25% stenosis) in the coronary angiograph; 2) patients with stable angina pectoris (SAP) showed a long-term and stable effort angina that had lasted for at least 3 months, and a positive stress test; 3) patients with unstable angina pectoris (UAP) showed either angina with a progressive crescendo pattern or angina that occurred at rest without a recent myocardial infarction; 4) the ECG of the patients showed transient ST-T segment depression and T-wave inversion, without significantly elevated levels of cardiac enzymes; and 5) patients with acute myocardial infarction (AMI) showed typical angina associated with ST-segment elevations in ECG, and the frequency of occurrence of elevated levels of creatine kinase and troponin-I in sera from those patients was more than three.

The exclusion criteria were non-cardiac diseases including acute or chronic infections, malignancies, autoimmune diseases, hyperthyroidism, and medication with immunosuppressive agents. The controls comprised 219 unrelated healthy people [age = 61.1 ± 5.8 years (mean ± SD)] who had undergone health examinations in the Medical Examination Center of our hospital. Control subjects were only included if they had no personal history of CAD, and we matched frequency to cases by gender and age; all participants were unrelated ethnic Han Chinese. The study was approved by the Human Research and Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University (Xinxiang, China), and was conducted according to all current ethical guidelines. All participants recruited to this study provided written informed consent.

Genotyping

Peripheral blood was drawn from a vein into a sterile tube containing ethylenediaminetetraacetic acid (EDTA). Plasma samples were stored at -20°C. Genomic DNA was extracted from frozen peripheral blood samples using a QIAamp Blood Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer instructions. Genotyping was performed using a SNaPshot SNP genotyping assay (Genesky, Shanghai, China). A touch-down polymerase chain reaction (Td-PCR) was carried out in a 20-μL reaction mixture. Next, a SNaPshot multiplex single-base extension reaction was performed in a 10-μL reaction volume. After purification using 1 U SAP for 60 min at 37°C followed by 15 min at 75°C, the extension reaction product was separated by capillary electrophoresis (ABI 3130XL Genetic Analyzer, Applied Biosystems Co. Ltd., California, USA), and the results were analyzed using the GeneMapper 4.1 software (Applied Biosystems Co. Ltd.). Fifty samples were selected randomly to repeat genotyping for quality control and the repeat accuracy was 100%.

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) for each SNP was assessed using the

GENEPOP software (version 4.0). A logistic regression analysis was conducted to calculate the adjusted odds ratios (ORs) and 95% confidence intervals (95%CIs) for associations between the genetic polymorphisms and CAD risk. Pairwise linkage disequilibrium (LD) statistics (D' and r^2) and haplotype frequency were calculated, and haplotype blocks were constructed using the Haploview software (version 4.0). To ensure that the LD blocks most closely reflected the population level LD patterns, the definitions of the blocks were based on the control samples alone. All data were analyzed using the SPSS software (version 20.0) (SPSS Inc., Chicago, IL, USA).

RESULTS

The distribution frequencies of the five genotyped SNPs were in agreement with HWE. LD analyses of the data for the patients and controls revealed that four SNPs (rs8193036, rs3819024, rs2275913, and rs8193037) were located in block 1 (Figure 1).

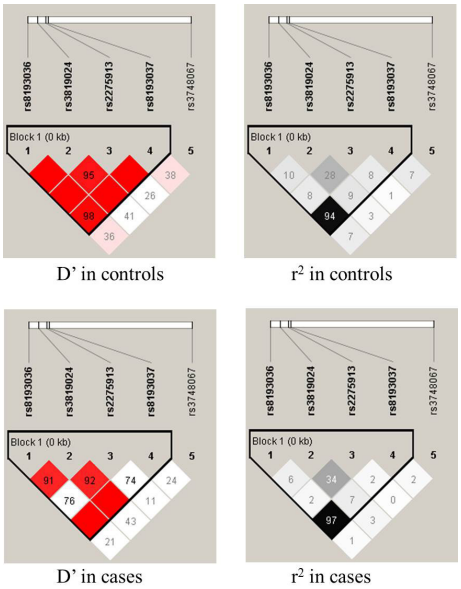

Figure 1. Linkage disequilibrium (LD) plot of the five single nucleotide polymorphisms (SNPs) in the *IL17A* gene. Values in squares are the pairwise calculations of r^2 (left) or D' (right). Black squares indicate $r^2 = 1$ (i.e., perfect LD between a pair of SNPs). Empty squares indicate D' = 1 (i.e., complete LD between a pair of SNPs).

The difference in the distribution of genotype frequencies of rs8193037 between CAD subjects and healthy controls was significant (P = 0.007). The CAD subjects had a significantly lower frequency of the A allele (P = 0.009, OR = 1.772, 95%CI = 1.146-2.742). There was a significant between-group difference in the genotype distribution of rs8193036 (P = 0.008). The CAD subjects had a significantly lower frequency of the T allele of rs8193036 (P = 0.010, OR = 1.754, 95%CI = 1.139-2.701) (Table 1).

Strong linkage disequilibrium was observed in one block (D' > 0.9). Significantly fewer T-G-G-A haplotypes (P = 0.045) were found in CAD subjects in block 1 (Table 2).

Table 1. Genotypic and allelic frequencies of *IL17A* polymorphisms in the controls and patients with coronary artery disease (CAD).

Variable	MAF	CAD (N = 219)		Controls (N = 219)		HWE	P value	OR (95%CI)
		N	%	N	%			
rs3819024	0.384					0.823	0.217	
GG		67	30.6	84	38.4		0.088	0.708 (0.477-1.053)
AG		112	51.1	102	46.6		0.339	1.201 (0.825-1.747)
AA		40	18.3	33	15.1		0.370	1.260 (0.760-2.086)
G allele		246	56.2	270	61.6		0.099	0.797 (0.609-1.044)
A allele		192	43.8	168	38.4			
rs2275913	0.336					0.613	0.977	
GG		93	42.5	95	43.4		0.847	0.963 (0.660-1.407)
AG		102	46.6	101	46.1		0.924	1.019 (0.700-1.483)
AA		24	11.0	23	10.5		0.877	1.049 (0.573-1.921)
G allele		288	65.8	291	66.4		0.830	0.970 (0.733-1.283)
A allele		150	34.2	147	33.6			
rs8193037	0.137					0.228	0.007	
GG		186	84.9	161	73.5		0.004	2.030 (1.261-3.270)
AG		30	13.7	56	25.6		0.002	0.462 (0.283-0.754)
AA		3	1.4	2	0.9		0.655	1.507 (0.249-9.108)
G allele		402	91.8	378	86.3		0.009	1.772 (1.146-2.742)
A allele		36	8.2	60	13.7			
rs8193036	0.139					0.205	0.008	
CC		185	84.5	160	73.1		0.004	2.006 (1.251-3.217)
TC		31	14.2	57	26.0		0.002	0.469 (0.288-0.761)
TT		3	1.4	2	0.9		0.655	1.507 (0.249-9.108)
C allele		401	91.6	377	86.1		0.010	1.754 (1.139-2.701)
T allele		37	8.4	61	13.9			
rs3748067	0.228					0.873	0.460	
AA		142	64.8	130	59.4		0.238	1.263 (0.858-1.859)
AG		69	31.5	78	35.6		0.363	0.832 (0.559-1.237)
GG		8	3.7	11	5.0		0.483	0.717 (0.283-1.818)
A allele		353	80.6	338	77.2		0.214	1.229 (0.887-1.701)
G allele		85	19.4	100	22.8			

MAF = minor allele frequencies; HWE = Hardy-Weinberg equilibrium.

Table 2. *IL17A* haplotype frequencies in block 1 and the results of their associations with risk of coronary artery disease (CAD).

Haplotype	Cases [N (%)]	Controls [N (%)]	Statistics		
			P	OR	95%CI
C-A-G-G	93 (42.466)	82 (37.443)	0.283	1.233	0.841-1.809
C-G-A-G	72 (32.877)	72 (32.877)	1.000	1.000	0.671-1.490
C-G-G-G	33 (15.068)	33 (15.068)	1.000	1.000	0.592-1.688
T-G-G-A	17 (7.763)	30 (13.699)	0.045	0.530	0.283-0.993

DISCUSSION

IL-17A is the most important member of the IL-17 family. The gene that encodes it (*IL17A*) has the chromosomal locus 6q12 and comprises three exons and two introns. Two previous studies have reported an association between the three common *IL17A* gene SNPs rs2275913, rs3819024, and rs3748067 and the risk of cardiovascular disease (Pei et al., 2009; Zhang et al., 2011). In this study, we performed a comprehensive analysis of the functional genetic variations of the *IL17A* gene, and identified two variants (rs8193037 and rs8193036) that are significantly associated with CAD risk in the Chinese Han population.

Zhang et al. (2011) studied five *IL17A* polymorphisms (rs4711998, rs3819024,

rs2275913, rs8193037, and rs3819024) in 1031 CAD patients and 935 healthy controls. The results indicated that the rs8193037 *IL17A* SNP is significantly associated with CAD risk in the Chinese Han population and the rs8193037 G allele is associated with increased expression of IL17-A in AMI patients, and may be an independent predictive factor for CAD. We observed fewer rs8193037 A allele carriers in the CAD patients than in the control subjects. The genetic evidence provided by the present study supports the biological relevance of IL-17A in CAD (Eid et al., 2009).

A previous study suggested that G homozygotes had increased plasma IL-17A levels compared with the A allele carriers in both cases and control subjects (Zhang et al., 2011). These findings indicate that the A allele of the rs8193037 SNP, which is associated with increased expression of IL-17A, might have a dangerous effect on CAD. The functional importance of this polymorphism, however, requires further studies.

Significant differences were also found in the distribution of allele frequencies of the rs8193036 SNP between the patients with CAD and the healthy controls. The frequency of the T allele of rs8193036 in the patients with CAD was significantly lower than in the controls. To the best of our knowledge, this is the first demonstration of the association between the rs8193036 T allele in the *IL17A* gene and susceptibility to CAD. Despite the fact that the precise molecular mechanism underlying our observations is unclear, one possible explanation is that this variant of the *IL17A* gene may lead to altered gene expression in CAD. The possible specific role of the functional SNPs reported in our study requires further exploration.

We further investigated the genetic interactions among polymorphisms and a strong linkage disequilibrium was observed. The haplotype analysis revealed that significantly more T-G-G-A haplotypes (block 1) were found in the controls, suggesting that they may protect against CAD. To some extent, this finding further supports a role of *IL17A* polymorphisms in CAD, while differences between ethnic groups may exist.

In summary, our study demonstrates the association between certain *IL17A* polymorphisms of the promoter region and CAD. The four *IL17A* polymorphisms were in high linkage disequilibrium and one haplotype was significantly associated with premature CAD risk. These findings encourage future research into functional polymorphisms within and close to the *IL17A* gene using a systemic approach in a larger sample population.

Conflicts of interest

The authors declare no conflict of interest.

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