

## Original Article

# Interleukin-16 gene polymorphism is associated with acute coronary syndrome in the Chinese Han population

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**Abstract:** Acute coronary syndrome (ACS) is clinical syndromes caused by coronary atherosclerosis plaque rupture or attack, which can lead to complete or incomplete pathological basis of occlusive thrombosis. Evidences have indicated the importance of gene polymorphism in the pathological progression of coronary heart disease risk. The purpose of this study was to investigate the associations between polymorphisms in interleukin-16 (IL-16) single nucleotide polymorphisms (SNP) and the risk of ACS in Chinese Han population. A total of 238 patients and 178 healthy individuals were recruited in this retrospective cohort. Genotyping of IL-16 rs8034928, rs3848180, rs1131445, rs4778889 and rs11556218 SNPs was performed by PCR-restriction fragment length polymorphisms. Results showed that the G allele in rs3848180 and C allele in rs1131445 were higher frequencies in ACS group than healthy group. We found that G/G genotype in rs1131445 showed a significant higher risk of CAS (OR=1.28, 95% CI=0.94-2.55). Variant of rs3848180 presented a significant increased risk of CAS in dominant (OR=1.44, 95% CI=1.04 -2.68). The allele C demonstrated a significant risk of CAS (OR=1.26, 95% CI=1.12-2.08) and T/T in rs3848180 were found to be associated with increasing risk of CAS (OR=1.66, 95% CI=1.07-4.25). In conclusion, these results showed that rs1131445 and rs3848180, SNPs of *IL-16* are associated with CAS risk in Chinese Han population, which provide the possibility of statistical power calculation to predict the susceptibility of ACS.

**Keywords:** Acute coronary syndrome, interleukin-16, SNP, Chinese Han population

## Introduction

Coronary heart disease (CAD) is one of the most common complicated cardiovascular diseases caused by atherosclerosis and vascular cavity stenosis or occlusion [1, 2]. Acute coronary syndrome (ACS) is one kind cardiovascular disease CVD disease caused by coronary atherosclerosis plaque rupture or attack, which can lead to complete or incomplete pathological basis of occlusive thrombosis, resulting in occurrence of CAD [3, 4]. Clinical manifestations of ACS include arrhythmia, heart failure, and even sudden death for patients [5, 6]. CAS also presents the highest mortality diseases in the world that closely associated with metabolism disorders of endogenous substances [7]. In recent years, genetics research has indicated the associations between gene polymorphism and the risk of CAS [8-11]. Findings have achieved advances in single nucleotide polymorphisms (SNP) in the pathological progression of CAD.

Interleukin-16 (IL-16) is a proinflammatory and immunoregulatory cytokine and composed by 631-amino acid [12]. Previous study has showed that IL-16 is generated by CD8+ cells, which lead to chemotaxis of CD4+, mononuclear cells and eosinophils as well as induce IL-2R and HLAII expression in T and mononuclear cells [13]. Many reports have investigated the genetic polymorphism of IL-16 and risk of human carcinoma, autoimmune diseases and CAD [14-16]. A study has indicated that The T/G TG and GG genotypes in IL-16 polymorphism gene rs-11556218 were associated with significantly decreased risk of chronic hepatitis B compared with the TT genotype, which also revealed that subjects with the G allele appeared to have lower susceptibility to chronic hepatitis B than those with the T allele [17]. Another study have showed that no association of IL-6 gene polymorphism (-174 G/C) with myocardial infarction or traditional cardiovascular risk factors [18]. However, no reports have analyzed the relationship between *IL-6* gene polymorphism and the risk of CAS.

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**Table 1.** The clinical characteristics of ACS patients and healthy individuals

Characteristic	ACS, N (%)	Health, N (%)	P value
Age-years	47.8±12.4	46.2±13.5	0.680
Male	120 (50.42)	92 (51.69)	0.0240
Female	118 (49.58)	86 (48.31)	0.0164
Hypertension	60 (25.22)	18 (10.11)	0.0024
Diabetes	32 (13.45)	10 (5.62)	0.0052
Smoking	88 (26.83%)	24 (13.48)	0.0018
TC	4.2±1.2	4.6±1.3	0.0344
TG	2.0±1.3	1.8±1.0	0.840
HDL-C	2.6±1.1	2.8±1.0	0.0382
LDL-C	2.9±0.8	41.8±0.5	0.00024

**Table 2.** Primer sequences and restriction endonucleases used for RFLP analysis

IL-16 SNP	Primers (5'-3')	Restriction endonuclease
rs8034928	F, 5'-TTCCATTGAAGAGAGC-3' R, 5'-TGCAGAAAACCCAGGTTTC-3'	Mae III
rs3848180	F, 5'-CCTCCAGTTCACAGCATCA-3' R, 5'-GCTCTACGTTAGTCCCTTA-3'	NcoI
rs1131445	F, 5'-TTGATGTTGGCTGGGAACT-3' R, 5'-CACGCTTTGAGCTTGGTG-3'	HinfI
rs4778889	F, 5'-CTCCACACTCAAAGCCTTT-3' R, 5'-CCATGTCAAACGGTAGCCT-3'	Spe I
rs11556218	F, 5'-CTCAGTTCACAGAGTGT-3' R, 5'-TGTGACAATCACAGCTTGCC-3'	BamH I

In this study, we investigated the role of polymorphism in IL-16 (rs8034928, rs3848180, rs1131445, rs4778889 and rs11556218) on the susceptibility of CAS in a retrospective cohort in Chinese Han population. Our results demonstrated that IL-6 SNP is likely to play a predictive role in the progression of CAS.

### Materials and methods

#### Study design, subjects and sampling

A total of 238 patients and 178 healthy individuals were included in this retrospective cohort in Chinese Han population (Xian province, China). The age was 47.8±12.4 and 46.2±13.5 years in ACS patients and healthy individuals, respectively. Inclusion criteria for individuals with ACS were diagnosed by angiographic evidence of ≥ 70% stenosis of one major coronary artery and/or ≥ 50% of the left main coronary

artery. The number of male and female in ACS patients (120 males and 118 females) and healthy individuals (92 males and 86 females) groups were approximate equal. The characteristics of patients with ACS and healthy volunteers were summarized in **Table 1**. The frequencies of *IL-16* gene polymorphism in ACS patients were designed in this retrospective pilot case-control study. This study was approved by the ethics committee of the first affiliated hospital of Xi'an Jiaotong University. All patients were asked to provide 5 ml venous blood and were required to write informed consent with signature.

#### DNA genotyping

All candidates loci of IL-16 for tag SNPs based on NCBI dbSNP database and SNP info and five SNPs rs1131445, rs3848180, rs4778889 and rs11556218, were selected, which is common variants with the minor allele frequency should ≥ 10% in the Chinese population. The genomic DNA was 10 µg of genomic DNA were isolated from extracted by the method of buffy-coat fractions with TIANamp blood DNA kit (Tiangen Biotech, Beijing, China) (50 ng of genomic DNA, 200 µM dNTP, 2.5 units of Taq DNA polymerase, and 200 µM primers) and used for PCR amplification followed preliminary denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, annealing temperature reduced to 64°C for 30 s, and 72°C for 10 min by volume of 20 µl containing 50 ng of genomic DNA, 200 µM dNTP, 2.5 units of Taq DNA polymerase, and 200 µM primers. PCR primers were designed using Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA) (**Table 2**). Genotyping of *IL-16* was conducted by PCR-restriction fragment length polymorphisms (RFLP) (**Supplementary Figure 1**) as described previously [19, 20].

#### ELISA

Serum levels of IL-16 (MBS700340, Thermo Fisher Scientific), were analyzed in patients with ACS and healthy individuals using ELISA kit according to the manufacturer's instrument.

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**Table 3.** Distribution of *IL-16* genes polymorphisms between ACS patients and healthy individuals

IL-16 SNP	Major/Minor allele	MAF	Minor frequencies		ACS (%)			Healthy (%)		
			ACS	Healthy	A/A	A/a	a/a	A/A	A/a	a/a
rs3848180	T/C	0.327	0.404	0.322	46	34	20	72	18	10
rs1131445	T/G	0.477	0.465	0.403	23	45	48	65	28	7
rs8034928	T/C	0.346	0.366	0.320	64	26	8	63	27	10
rs4778889	C/G	0.315	0.308	0.298	42	44	12	40	42	18
rs11556218	G/T	0.204	0.212	0.207	56	30	14	54	26	20

**Table 4.** Genotype, allele frequency *IL-16* gene polymorphisms between ACS patients and healthy individuals

IL-16 SNP		Minor allele frequencies (%)		OR (95% CI)		
		ACS	Healthy	Codominant	Dominant	Recessive
rs3848180	C/T	35.2	36.1	-1.12 (0.94-1.86)	-1.02 (0.82-1.48)	-
	T/T	76	42	1.44 (1.04-2.68)	-1.14 (0.74-1.26)	-
	G/G	41.3	45.6	-0.84 (0.54-1.26)	-1.22 (0.82-1.75)	-
rs1131445	C/T	72	68	-0.70 (0.36-0.95)	-0.80 (0.56-1.47)	-
	T/G	43.2	45.2	1.04 (0.66-2.18)	1.34 (1.25-2.68)	1.62 (1.12-4.28)
	G/G	94	75	1.28 (0.94-2.55)	-1.13 (0.58-1.92)	-

The serum concentration levels of IL-16 were measured by an enzyme micro-plate reader at 450 nm.

### Statistical analysis

Continuous variables were shown as mean  $\pm$  SD and analyzed by students t test. All data were analyzed using SPSS Statistics 19.0 and Graphpad Prism version 5.0 with the help of Microsoft Excel. Allele and genotype frequencies were calculated in each group (patients and healthy individuals) using direct counting. Hardy-Weinberg equilibrium (HWE) and the differences between allele and genotype frequencies were calculated using Fisher's exact test or Chi-square test. 95% confidence intervals (CI) and odds ratios (OR) were presented using logistic regression models to assess the magnitude of association between SNPs and clinical groups compare to healthy individuals. Results of allele and genotype frequencies were determined by STATA SE 12.1 software. Minor allele frequencies (MAF) were calculated using the prop.test function on R 3.1.1 software. \* $P < 0.05$  was considered statistical differences.

### Results

A total of 238 patients and 178 healthy individuals were included in this retrospective cohort in Chinese Han population. The mean of

age was  $47.8 \pm 12.4$  and  $46.2 \pm 13.5$  years in ACS patients and healthy individuals, respectively. The number of male and female ACS patients (120 males and 118 females) and healthy individuals (92 males and 86 females) were approximate equal. The proportion of smoking status and diabetes were higher in CAS patients. The characteristics of CAS patients and healthy individuals were summarized in **Table 1**. We observed that TC, HDL-C and LDL-C levels were lower in ACS patients than healthy individuals. Results showed that IL-16 serum levels were up-regulated in ACS patients compared with healthy individuals.

We analyzed five SNPs genotype distributions in *IL-16* gene (rs8034928, rs3848180, rs1131445, rs4778889 and rs11556218) in ACS patients and healthy individuals (**Table 3**). Results showed that rs1131445 and rs3848180 genotype in *IL-16* gene is more frequency in ACS patients than healthy individuals ( $P < 0.01$ ). We did not observe any significant difference between the genotype frequencies of *IL-16* rs8034928, rs4778889 and rs11556218 ( $P > 0.05$ ).

We further analyzed the effect of *IL-16* rs-1131445 and rs3848180 polymorphisms on the ACS risk using Multivariate logistic regression analysis (**Table 4**). We showed that the C allele in rs1131445 and G allele in rs3848180

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**Table 5.** Combined effects of *IL-16* SNPs rs1131445 and rs3848180 on the ACS risk

<i>IL-16</i> SNPS		ACS (n, %)	Healthy (n, %)	OR (95% CI)
rs1131445	rs3848180			
GG	TT	54 (14.2)	40 (45.6)	2.68 (1.22-4.32)
C	TT	54 (21.3)	38 (40.8)	1.90 (0.82-2.85)
GG	G	45 (24.2)	32 (23.8)	-1.28 (0.74-1.86)
C	G	40 (18.6)	36 (33.6)	1.78 (0.82-2.60)

were higher frequencies in ACS group than healthy group. Variant of T/T rs3848180 presented a significant increased risk of CAS in dominant model (OR=1.44, 95% CI=1.04-2.68). Frequencies of C/T and G/G in rs3848180 polymorphisms showed no significant difference between ACS patients and healthy individuals ( $P>0.05$ ). We found that T/T genotype in rs3848180 showed a significant higher risk of CAS in dominant model (OR=1.28, 95% CI=0.94-2.55). No significant differences of C/T and G/G in rs3848180 were observed between ACS patients and healthy individuals.

We finally investigated the cooperated effects of *IL-16* gene rs1131445 and rs3848180 on the ACS risk determined by multivariate logistic regression analysis (Table 5). Results demonstrated that allele C in rs1131445 and T/T genotype in rs3848180, rs1131445 C and rs3848180 G genotypes presented a higher risk of ACS. Notably, we also found that G/G in rs1131445 and T/T genotype in rs3848180 showed the highest risk of CAS than C-TT and C-G single wild-type genotypes (OR=2.74, 95% CI=1.60-3.78). However, G in rs1131445 and GG genotype in rs3848180 showed lower risk of CAS.

### Discussion

ACS refers to a group of clinical heart disease caused by myocardial ischemia [21]. Researches have showed that genetic polymorphism of *IL-16* is associated with multiple human diseases [22-24]. In the current study, we investigated the frequency of SNPs in *IL-16* gene in ACS patients. Polymorphisms rs8034928, rs3848180, rs1131445, rs4778889 and rs11556218 in *IL-16* gene have shown associations with the risk of human ACS. Findings have indicated that rs3848180 and rs1131445, SNPs of *IL-16* are associated with CAS risk in Chinese Han population, which provide the possibility of

statistical power calculation to predict the susceptibility of CAS.

Recently, the functions of single nucleotide polymorphisms for the diagnosis and progression and of CAD have been widely investigated in different genes, which related the risk or predictor of human CAD [25, 26]. It has attracted increasing interest of investi-

gators to identify novel genetic variants for evaluating the early risk and pathological progression of CAD [27-29]. In this study, we further investigated the role of inflammatory cytokine *IL-16* gene polymorphism in disease etiology of ACS based on the genetic information.

Previous study has indicated that endarterectomy patients with elevated levels of circulating *IL-16*, who had fewer cardiovascular events during follow-up [30]. In addition, the association between *IL-16* polymorphism and risk of renal cell carcinoma: association in a Chinese population [31]. Furthermore, the roles of *IL-16* SNPs rs8034928 and rs3848180 in human disease have been analyzed and results indicated that the genotype and allele frequencies of the rs4778889 T/C polymorphism were statistically different between patients with endometriosis and controls, resulting in a significantly increased proportion of TC heterozygote and CC homozygote carriers among patients with endometriosis [13, 32]. Results in the current study have indicated that the G allele in rs3848180 and C allele in rs1131445 were higher frequencies in ACS group than healthy group. We found that C/C genotype in rs1131445 showed a significant higher risk of CAS (OR=1.28, 95% CI=0.94-2.55).

Although previous report has suggested polymorphisms of *IL-16* rs11556218 and rs8034928 are associated with risk of CAD [33], the role of *IL-16* SNPs rs8034928, rs3848180, rs1131445, rs4778889 and rs11556218 were firstly reported in patients with ACS. Findings in this study also support the association between polymorphism of *IL-16* rs3848180, rs1131445 SNPs and the risk of ACS. We also investigated the cooperated effects of *IL-16* gene rs8034928 and rs3848180 on the ACS risk and demonstrated that allele C in rs1131445 and G/G genotype in rs3848180 presented a higher risk of ACS rs1131445 C/C and rs3848180 C/T genotypes.

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In conclusion, this study showed the variants of IL-16 rs1131445, and rs3848180 SNPs are associated with risk of ACS. Findings indicated C in rs1131445 and G/G genotype in rs3848180 showed a higher risk of CAS than G/G and C double wild-type genotypes. These results suggest that IL-16 SNPs may be used as a predictive molecular to evaluate the risk of ACS. However, further study should be confirmed our results since multiple genes and environmental factors involves in the pathological processes of ACS.

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### Disclosure of conflict of interest

None.

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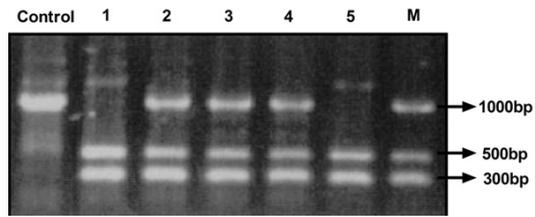
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**Supplementary Figure 1.** Genotyping of *IL-16* determined by PCR-restriction fragment length polymorphisms (RFLP). PCR-restriction fragment length polymorphism analysis of *IL-16* in the acute coronary syndrome patients in the Chinese Han population. Polymorphisms were obtained by digestion of PCR products with *Spe I* restriction enzyme followed by resolution with agarose gel electrophoresis.