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Jie Lou, QianZhen Huang, Runfeng Zhan & Qiulan Yang

The effect of inflammatory factors on unstable angina risk, from the gene level

Jie Lou[1#], QianZhen Huang[1#], Runfeng Zhang[2, 3*], Qiulan Yang[1*]

[1]Department of Clinical Laboratory, Taicang TCM Hospital Affiliated to Nanjing University of Chinese Medicine, Suzhou, 215006, China

[2]Department of Cardiovascular Medicine, School of Clinical Medicine, Southwest Medical University，Luzhou, 646000, Sichuan, China

[3]Department of Cardiovascular Medicine, Mianyang 404 Hospital, Mianyang, 621000，Sichuan, China

[*]**Corresponding authors:** Qiulan Yang at Department of Clinical Laboratory, Taicang TCM Hospital Affiliated to Nanjing University of Chinese Medicine, No.140 renminnan road, Taicang, Jiangsu Province, China. Email: Yang18051238167@163.com. Runfeng Zhang at Department of Cardiovascular Medicine, School of Clinical Medicine, Southwest Medical University，Luzhou, 646000, Sichuan, China and at Department of Cardiovascular Medicine, Mianyang 404 Hospital,Mianyang,621000, Sichuan, China. Email: stephenstewart@yeah.net.

[#]Equal contributors and co-first authors.

Abstract

Background: Inflammation and its genetic regulation play crucial roles in the development of unstable angina (UA), a key manifestation of coronary artery disease (CAD). However, the genetic determinants that influence cytokine production and interindividual inflammatory responses remain incompletely understood, particularly in the Chinese population. This study aimed to clarify the relationship between inflammatory cytokine gene polymorphisms, serum cytokine expression, and UA risk.

Methods: A hospital-based case–control study was conducted in 160 patients with UA and 280 age- and sex-matched healthy controls. Participants meeting the 2007 ACC/AHA UA/NSTEMI diagnostic criteria and angiographic evidence of $\geq 50\%$ coronary stenosis were included, while individuals with systemic inflammatory or metabolic disorders were excluded. Serum cytokine levels (IL-1β, IL-6, IL-10, IL-17, IFN-γ, TNF-α) were quantified by ELISA, and 13 cytokine gene loci were genotyped using PCR-RFLP. Associations between polymorphisms and UA were evaluated using multivariate logistic regression with odds ratios (ORs) and 95% confidence intervals (CIs).

Results: All cytokines were significantly elevated in UA patients compared with controls ($p < 0.01$), with IL-10 showing the largest increase (3.2-fold). Polymorphisms at seven loci were significantly associated with UA susceptibility: IL-1β -511 C/T (TT vs CC: OR =

6.39, 95% CI 2.94–13.87), TNF-α −308 G/A (AA vs GG: OR = 2.56, 95% CI 1.50–4.37), IL-6 −1363 G/T (GT vs GG: OR = 0.52, 95% CI 0.34–0.80), IL-10 −1082 G/A and −592 C/A, IL-17 rs2275913, and IFN-γ +874 A/T (all $p < 0.05$). ROC analysis revealed strong discriminatory ability for IL-1β −511 C/T (AUC = 0.87) and TNF-α −308 G/A (AUC = 0.84), both with sensitivity and specificity > 80%. Moreover, carriers of risk alleles (e.g., IL-1β T, TNF-α A) exhibited higher cytokine levels, indicating functional upregulation of inflammatory activity.

Conclusion: Cytokine gene polymorphisms, particularly IL-1β −511 C/T and TNF-α −308 G/A, are associated with heightened inflammatory responses and increased UA susceptibility. These variants may serve as potential genetic biomarkers for coronary instability, though further multicenter and functional validation is warranted before clinical application.

Keywords: unstable angina; coronary artery disease; inflammatory cytokines; genetic polymorphism; biomarker; Enzyme-Linked Immunosorbent Assay; Polymerase Chain Reaction–Restriction Fragment Length Polymorphism

Introduction

Coronary heart disease (CHD) represents a classic multifactorial disorder, wherein the intricate interplay of genetic predisposition, metabolic anomalies, and environmental exposures collectively propels the progression of atherosclerosis and ensuing vascular dysfunction [1]. While established risk factors such as hypertension,

dyslipidemia, and smoking significantly contribute to its onset, the underlying pathological cornerstone remains atherosclerosis [1]. Accumulating evidence underscores the substantial role of genetic susceptibility in modulating these risk factors, particularly pathways related to oxidative stress and endothelial integrity [2]. Consequently, molecular genetic investigations have pinpointed a growing number of candidate genes implicated in CHD susceptibility [3-5]. However, the reproducibility of these genetic associations often varies across different studies, highlighting the critical need to examine population-specific genetic architectures to fully elucidate CHD pathogenesis. Notably, contemporary national surveillance in China reported that the prevalence of CHD among residents aged ≥18 years was 758 per 100,000 (including acute myocardial infarction, PCI/CABG, and hospitalizations for unstable angina), and GBD-based estimates suggest an age-standardized CHD incidence of ~197.4 per 100,000 person-years in 2019, underscoring the substantial clinical burden and the need for Chinese population–specific genetic evidence [4].

Unstable angina (UA) marks an acute and critical transitional phase within the CHD spectrum. It is primarily characterized by plaque instability, rupture, and subsequent thrombosis, culminating in myocardial ischemia [6, 7]. Central to these pathogenic events is the activation of inflammatory cascades, which promote endothelial dysfunction, facilitate leukocyte infiltration, and exacerbate oxidative damage [6, 7]. Key proinflammatory cytokines, including IL-1β, IL-6, TNF-α, and IFN-γ, have been identified as crucial mediators, participating not only in the initial formation of atherosclerotic plaques but also in their eventual destabilization, thereby triggering acute coronary syndromes [6, 7].

Notwithstanding the well-documented role of inflammation in atherosclerosis and UA, the specific genetic determinants that govern interindividual variability in cytokine production and inflammatory responses remain incompletely elucidated. Previous research has reported associations between certain cytokine gene polymorphisms—such as those in TGF-β1 and its receptor [8], as well as the TNF-α (-308 G/A) variant [9, 10]—and the risk of coronary syndromes. These findings suggest that specific allelic variants can modulate disease susceptibility by fine-tuning inflammatory signaling. Nonetheless, the majority of these studies have been conducted in European or Middle Eastern populations, and their findings may not be directly generalizable to other ethnic or geographical groups, due to distinct genetic backgrounds and environmental influences. To date, systematic investigations into the relationship between inflammatory cytokine gene polymorphisms and UA risk in Chinese populations, particularly among individuals from Southwest China, remain scarce.

Therefore, building upon prior evidence that links cytokine polymorphisms to coronary syndromes [8-10], we hypothesize that functional genetic variations in pivotal inflammatory cytokine genes—namely IL-1β, IL-6, IL-10, IL-17, IFN-γ, and TNF-α—are associated with altered susceptibility to UA in the Southwest Chinese population. This study aims to quantitatively assess serum cytokine expression levels and rigorously analyze their relationship with these specific gene polymorphisms. Our objective is to clarify the genetic contribution of inflammatory pathways to UA pathogenesis. By focusing on this underrepresented regional population, we anticipate that our findings will provide novel insights into the molecular mechanisms underpinning coronary

instability and potentially inform future strategies for precision prevention.

Materials and methods

Study Population and Eligibility Criteria

This prospective case-control study was conducted at our Hospital (a tertiary Grade-A general hospital) between January 2023 and January 2024, enrolling 160 patients with unstable angina (UA) and 280 age- and sex-matched healthy controls. All UA patients received standard anti-ischemic therapy, including aspirin, statins, and β-blockers as appropriate. Inclusion criteria were as follows: (1) Clinical diagnosis of UA according to the 2007 ACC/AHA UA/NSTEMI Guidelines (criteria concordant with later updates, including the 2014 AHA/ACC NSTE-ACS guideline and the 2021 AHA/ACC chest pain guideline); (2) Angiographic confirmation of ≥50% diameter stenosis in ≥1 subepicardial coronary artery or major branch.

Exclusion criteria were defined to minimize confounding influences on inflammatory and genetic parameters. Specifically, individuals with cardiometabolic disorders (e.g., diabetes mellitus or familial hypercholesterolemia [LDL-C >190 mg/dL]) and advanced cardiac dysfunction (NYHA class II–IV heart failure) were excluded, since these conditions independently alter inflammatory cytokine expression and could obscure UA-specific effects. Systemic comorbidities, including chronic kidney disease (eGFR <60 mL/min/1.73 m^2), hepatic dysfunction (ALT/AST >3×ULN), autoimmune diseases, and hematologic malignancies, were also

excluded to avoid systemic inflammatory interference and to ensure patient safety during blood sampling. Proinflammatory confounders, such as acute infection (CRP >10 mg/L), recent surgery or thrombosis (≤3 months), or ongoing immunosuppressive therapy, were omitted to control for transient inflammatory changes unrelated to UA pathogenesis. Procedural factors (e.g., prior PCI or CABG) and connective tissue disorders that may affect vascular integrity were similarly excluded.

The control group comprised 280 age- and sex-matched healthy volunteers recruited from routine health examinations. All controls had no history of ischemic heart disease or angina-equivalent symptoms and exhibited normal ECG and echocardiographic findings. To exclude overt or subclinical inflammatory conditions, controls were additionally screened by medical history and physical examination and routine laboratory testing (complete blood count and C-reactive protein [CRP]); individuals with recent infection, chronic inflammatory/autoimmune diseases, or elevated CRP (>10 mg/L) were excluded.

Written informed consent was obtained from all participants following detailed protocol disclosure. The study protocol adhered to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Our Hospital.

Epidemiological investigation

A standardized epidemiological questionnaire was developed based on previous literature and established survey frameworks. The instrument was designed to ensure consistency in data collection across all participants. It included four main sections: (1) general information (age, sex, place of residence, clinical symptoms, and disease duration); (2) medical history (hypertension, coronary heart disease, diabetes, and hyperlipidemia); (3) lifestyle factors

(smoking and alcohol consumption); and (4) family history of cardiovascular or metabolic disorders.

All investigators underwent unified training to minimize interviewer bias and ensure accurate data recording. The questionnaire was pretested in 20 participants to evaluate clarity and reliability, with subsequent revisions based on feedback. Data entry was double-checked by two independent researchers to maintain consistency. Continuous variables were verified against medical records whenever available to improve accuracy.

Gene expression level of inflammatory factors

Morning fasting venous blood (5 mL) was collected into heparin sodium–anticoagulated tubes and centrifuged at 3,000 ×g for 15 minutes at 4°C to isolate serum. Aliquots were immediately stored at −80°C in RNA-free cryovials to minimize cytokine degradation. Serum concentrations of inflammatory cytokines (IL-1β, IL-6, IL-10, IL-17, IFN-γ, and TNF-α) were quantified using high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Shenko Biotechnology, China; catalogue numbers IL-1β: SEB972Hu, IL-6: SEB295Hu, IL-10: SEB170Hu, IL-17: SEB421Hu, IFN-γ: SEB552Hu, TNF-α: SEB862Hu).

All assays were performed in duplicate according to the manufacturer's protocol, with standard 5-point calibration curves ($R^2 \geq 0.99$). Quality control included internal standards and inter-assay coefficient of variation maintained below 15%. Sample analysis was conducted in a blinded manner by two independent technicians to minimize observer bias.

Gene polymorphism analysis

Genomic DNA was extracted from peripheral blood leukocytes using a commercial DNA isolation kit (Tiangen Biotech, Beijing,

China; catalogue no. DP304). Genetic polymorphisms across key cytokine loci were analyzed, including: IL-1β (−511 C/T, +3954 C/T, −31 C/T), IL-6 (−174 G/C, −1363 G/T), IL-10 (−592 C/A, −1082 G/A, −819 T/C), IL-17 (rs2275913, rs763780), IFN-γ (+874 A/T, +2108 A/G), and TNF-α (−308 G/A, −376 G/A).

Candidate genes and variants were chosen based on prior evidence implicating inflammatory cytokine pathways in atherosclerosis and acute coronary syndromes [8-10]. Each locus was previously reported to influence cytokine transcriptional activity or serum concentration, providing a mechanistic rationale for inclusion. This targeted candidate-gene approach aimed to validate specific, biologically relevant variants rather than conduct a broad genotyping screen.

Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) was performed using validated primer sequences (5′–3′) and expected amplicon sizes, which are provided in Supplementary Table S1. PCR amplification was carried out in a 25 μL reaction mixture containing 100 ng genomic DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.4 μM of each primer, and 1 U Taq DNA polymerase (Takara Bio, Japan). The cycling conditions were as follows: initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 30 s, primer-specific annealing temperature for 30 s, and 72°C for 45 s; followed by a final extension at 72°C for 10 min.

PCR products were digested with the appropriate restriction endonucleases (Thermo Fisher Scientific, USA) and separated by 3% agarose gel electrophoresis. Approximately 10% of samples were randomly re-genotyped for verification, yielding >99% concordance. Laboratory staff were blinded to case/control status during all genotyping and data analysis procedures.

Statistical analysis

All statistical analyses were performed using SPSS version 26.0 (IBM Corp., USA) and GraphPad Prism 9.0 (GraphPad Software, USA). Continuous variables were expressed as mean ± standard deviation (SD) and compared using Student's t-test or one-way ANOVA, as appropriate. Categorical variables were expressed as counts and percentages and compared using the χ^2 test or Fisher's exact test.

Genotype and allele frequencies were calculated by direct counting. Hardy–Weinberg equilibrium (HWE) was tested in the control group using the χ^2 test. Associations between cytokine gene polymorphisms and unstable angina (UA) risk were estimated by calculating odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression.

To address the potential inflation of type I error due to multiple comparisons across loci, the Bonferroni correction was applied to adjust significance thresholds ($\alpha = 0.05 / n$, where n is the number of independent loci analyzed). Sensitivity analyses were also conducted using the Benjamini–Hochberg false discovery rate (FDR) approach to confirm the robustness of results.

For loci in HWE, genotype–phenotype associations were evaluated under codominant (AA vs AB vs BB), dominant/recessive ((AA+AB) vs BB), and heterozygote advantage (AB vs AA+BB) models.

Sensitivity, specificity, and post-hoc statistical power were calculated for significant loci using ROC and power analysis.

All confounding variables were pre-specified based on clinical relevance prior to analysis, including age, sex, smoking status, hypertension, and lipid levels. Adjusted logistic regression models were constructed accordingly. Cytokine serum levels were analyzed by independent-sample t-tests or one-way ANOVA after assessing

normality using the Shapiro–Wilk test; non-parametric tests were used when appropriate. Two-sided p values <0.05 (after correction) were considered statistically significant.

3 Results

3.1 Clinical indicators

A total of 160 patients with UA and 280 control subjects were included in the analysis. The two groups were generally comparable in demographic and clinical characteristics (Table 1). The mean age was 68.8 ± 7.1 years in the UA group and 67.6 ± 6.8 years in controls (p = 0.08). Men accounted for 71.9% of the UA group and 68.6% of the control group (p = 0.47). Mean BMI values were similar between groups (24.8 ± 1.8 kg/m² vs. 24.6 ± 1.8 kg/m², p = 0.26).

Lifestyle factors such as current smoking (27.5% vs. 28.5%) and drinking history (20.6% vs. 19.6%) did not differ significantly between groups. Likewise, the prevalence of diabetes mellitus (20.0% vs. 18.6%) and hypertension (21.3% vs. 20.4%) was comparable.

Serum lipid profiles were also similar: triglyceride levels showed comparable medians [1.7 (1.3–2.1) mmol/L vs. 1.7 (1.2–2.0) mmol/L, p = 0.99], total cholesterol (4.8 ± 0.9 mmol/L vs. 4.7 ± 0.8 mmol/L, p = 0.23), LDL-C (3.3 ± 0.8 mmol/L vs. 3.2 ± 0.7 mmol/L, p = 0.17), and HDL-C (1.9 ± 0.6 mmol/L vs. 2.0 ± 0.7 mmol/L, p = 0.13).

Table 1 Participants' characteristics of UA group and control group

Characteristics	UA group (n = 160)	Control group (n = 280)	*p* value
Age (years)	68.8 ± 7.1	67.6 ± 6.8	0.08
Men, n (%)	115 (71.9%)	192 (68.6%)	0.47
BMI (kg/m²)	24.8 ± 1.8	24.6 ± 1.8	0.26
Current smokers,	44 (27.5%)	80 (28.5%)	0.33

n (%)			
Drinking history, n (%)	33 (20.6%)	55 (19.6%)	0.73
Diabetes mellitus, n (%)	32 (20.0%)	52 (18.6%)	0.83
Hypertension, n (%)	34 (21.3%)	57 (20.4%)	0.70
TG (mmol/L)	1.7 [1.3–2.1]	1.7 [1.2–2.0]	0.99
TC (mmol/L)	4.8 ± 0.9	4.7 ± 0.8	0.23
LDL-C (mmol/L)	3.3 ± 0.8	3.2 ± 0.7	0.17
HDL-C (mmol/L)	1.9 ± 0.6	2.0 ± 0.7	0.13

Abbreviations: UA, unstable angina; BMI, body mass index; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Continuous variables were tested for normality using the Shapiro–Wilk test. Normally distributed data are presented as mean ± SD; skewed data (e.g., TG) are presented as median [IQR]. Categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate. p-values are reported to two decimal places.

3.2 Cytokines expression level

As shown in Figure 1 and Table 2 (UA, n = 160; controls, n = 280), serum concentrations of inflammatory cytokines were significantly higher in patients with UA than in healthy controls ($p < 0.01$ for all comparisons). Specifically, IFN-γ levels were 16.8 ± 3.9 pg/mL in the UA group versus 9.7 ± 2.8 pg/mL in controls, representing approximately a 1.7-fold increase. TNF-α concentrations were 23.4 ± 6.7 pg/mL compared with 17.1 ± 5.2 pg/mL (≈1.4-fold). IL-1β levels were elevated from 5.6 ± 2.5 pg/mL in controls to 8.5 ± 3.6 pg/mL in UA patients (≈1.5-fold). Similarly, IL-6 showed a substantial rise (15.2 ± 4.3 vs. 8.5 ± 2.7 pg/mL; ≈1.8-fold), while IL-17 increased to 17.7 ± 3.1 pg/mL compared with 11.3 ± 2.2

pg/mL (≈1.6-fold). Notably, IL-10 displayed the largest relative elevation, reaching 17.1 ± 2.4 pg/mL in the UA group versus 5.4 ± 1.9 pg/mL in controls—an approximate 3.2-fold increase.

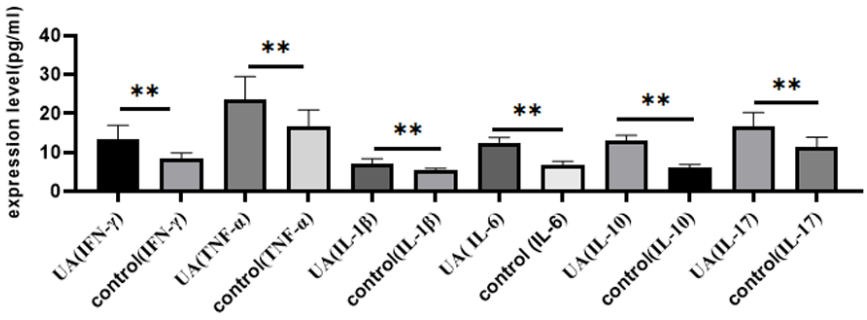

Figure 1 Serum cytokine expression

Table 2. Serum cytokine expression levels in the UA and control groups

Cytokine	UA group (pg/mL, mean ± SD)	Control group (pg/mL, mean ± SD)	*p* value
IFN-γ	16.8 ± 3.9	9.7 ± 2.8	< 0.01
TNF-α	23.4 ± 6.7	17.1 ± 5.2	< 0.01
IL-1β	8.5 ± 3.6	5.6 ± 2.5	< 0.01
IL-6	15.2 ± 4.3	8.5 ± 2.7	< 0.01
IL-10	17.1 ± 2.4	5.4 ± 1.9	< 0.01
IL-17	17.7 ± 3.1	11.3 ± 2.2	< 0.01

Abbreviations: UA, unstable angina; SD, standard deviation; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; IL, interleukin. Values are expressed as mean ± SD. All cytokine levels were significantly higher in the UA group compared with controls.

3.3 Gene Polymorphism Analyses

HWE analysis showed disequilibrium at four loci in the control group (IL-1β −511 C/T, IL-10 −592 C/A, IL-17 rs2275913, TNF-α −308 G/A) and two loci in the UA group, which may affect genotype-based comparisons; thus, subsequent analyses focused on allelic associations and Mendelian genetic models.

As summarized in Tables 3–5, several cytokine gene polymorphisms showed significant associations with UA risk after logistic regression adjustment. For the IL-1B gene, the -511 C/T polymorphism displayed a strong association with UA susceptibility. Compared with the CC genotype, both TC (OR = 5.02, 95% CI 2.21–11.49, $p < 0.001$) and TT (OR = 6.39, 95% CI 2.94–13.87, $p < 0.001$) genotypes conferred markedly increased risk, and the T allele itself was also associated with higher risk (OR = 2.52, 95% CI 1.81–3.50, $p < 0.001$). No significant associations were observed for IL-1B +3954 C/T or -31 C/T variants.

For TNF-α, carriers of the -308 A allele had a substantially elevated UA risk. Individuals with GA (OR = 2.01, 95% CI 1.23–3.27, $p = 0.007$) and AA (OR = 2.56, 95% CI 1.50–4.37, $p < 0.001$) genotypes exhibited higher susceptibility compared with GG carriers, while the A allele remained a significant risk factor (OR = 1.69, 95% CI 1.28–2.23, $p < 0.001$). No significant relationship was found for the -376 G/A polymorphism.

For IL-6, the -1363 G/T variant was protective: the GT genotype (OR = 0.52, 95% CI 0.34–0.80, $p = 0.004$) and the T allele (OR = 0.66, 95% CI 0.49–0.89, $p = 0.007$) were associated with reduced UA risk compared with the GG genotype, suggesting a possible down-regulatory role of the T allele. The -174 G/C polymorphism did not reach statistical significance.

Regarding IL-10, two promoter variants—-592 C/A and -1082 G/A—were significantly linked to UA. For -592 C/A, both heterozygous (CA, OR = 2.42, 95% CI 1.43–4.08, $p < 0.001$) and homozygous (AA, OR = 2.28, 95% CI 1.31–3.99, $p = 0.003$) genotypes conferred higher risk relative to CC carriers; the A allele was also positively associated (OR = 1.51, 95% CI 1.14–1.99, $p = 0.004$). Similarly, the -1082 A allele increased susceptibility (OR =

1.65, 95% CI 1.25–2.17, p < 0.001). No significant effect was found for -819 T/C.

For IL-17 rs2275913, both GA (OR = 2.16, 95% CI 1.41–3.31, p < 0.001) and AA (OR = 2.73, 95% CI 1.43–5.20, p = 0.003) genotypes increased UA risk compared with GG, and the A allele was associated with higher susceptibility (OR = 1.75, 95% CI 1.32–2.34, p < 0.001). No significant associations were observed for rs763780.

For IFN-γ +874 A/T, the TT genotype markedly increased risk (OR = 2.58, 95% CI 1.23–5.41, p = 0.017), while the A allele was slightly less frequent among UA patients (p = 0.084). The +2108 A/G variant showed no significant difference between groups. Full genotype distributions of non-significant loci are provided in Supplementary Table S2.

Consistent results were observed under codominant and dominant/recessive models (Table 6). For the most significant loci (e.g., IL-1β −511 C/T and TNF-α −308 G/A), ROC curves were constructed using genotype (coded under the best-fitting genetic model) as the predictor and case status (UA vs. controls) as the endpoint; sensitivity and specificity exceeded 80% with AUC values ≥ 0.84 (Figure 2), supporting their discriminative capacity for UA risk.

Table 3. Genotype and allele frequencies of pro-inflammatory cytokine genes (IL-1B and TNF-α) in UA and control groups

Gene / Locus	Genotype / Allele	Control n (%)	UA n (%)	OR (95% CI)[a]	*p* value
IL-1B −511 C/T					
	CC	66 (23.6)	8 (3.7)	1.00 REF	–
	TC	72 (25.7)	44 (27.5)	**5.02 (2.21–11.49)**	< 0.001
	TT	142 (50.7)	110 (68.8)	**6.39 (2.94–**	< 0.001

				13.87)	
	C allele	204 (36.4)	60 (18.8)	1.00 REF	–
	T allele	356 (63.6)	264 (81.2)	**2.52 (1.81–3.50)**	< 0.001
TNF-α −308 G/A					
	GG	102 (36.4)	33 (20.6)	1.00 REF	–
	GA	114 (40.7)	74 (46.3)	**2.01 (1.23–3.27)**	0.007
	AA	64 (22.9)	53 (33.1)	**2.56 (1.50–4.37)**	< 0.001
	G allele	318 (56.8)	140 (43.8)	1.00 REF	–
	A allele	242 (43.2)	180 (56.2)	**1.69 (1.28–2.23)**	< 0.001

Abbreviations: UA, unstable angina; OR, odds ratio; CI, confidence interval.

[a]Adjusted by logistic regression for age, sex, BMI, and smoking.

Table 4. Genotype and allele frequencies of regulatory cytokine genes (IL-6 and IL-10) in UA and control groups

Gene / Locus	Genotype / Allele	Control n (%)	UA n (%)	OR (95% CI)[a]	*p* value
IL-6 −1363 G/T	GG	115 (41.1)	90 (56.2)	1.00 REF	–
	GT	118 (42.2)	48 (30.0)	**0.52 (0.34–0.80)**	0.004
	TT	47 (16.8)	22 (13.8)	0.60 (0.34–1.06)	0.106
	G allele	348 (62.1)	228 (71.3)	1.00 REF	–
	T allele	212 (37.9)	92 (28.7)	**0.66 (0.49–0.89)**	0.007
IL-10 −592 C/A	CC	88 (31.4)	26 (16.3)	1.00 REF	–
	CA	112 (40.0)	80 (50.0)	**2.42 (1.43–4.08)**	< 0.001
	AA	80 (28.6)	54 (33.7)	**2.28 (1.31–3.99)**	0.003
	C allele	288 (51.4)	132 (41.3)	1.00 REF	–
	A allele	272 (48.6)	188 (58.7)	**1.51 (1.14–1.99)**	0.004

IL-10 −1082 G/A	GG	102 (36.4)	30 (18.8)	1.00 REF	–
	GA	114 (40.7)	82 (51.2)	**2.45 (1.49–4.02)**	< 0.001
	AA	64 (22.9)	48 (30.0)	**2.55 (1.47–4.43)**	< 0.001
	G allele	318 (56.8)	142 (44.4)	1.00 REF	–
	A allele	242 (43.2)	178 (55.6)	**1.65 (1.25–2.17)**	< 0.001

Abbreviations: UA, unstable angina; OR, odds ratio; CI, confidence interval.

[a]Adjusted by logistic regression for age, sex, BMI, and smoking.

Table 5. Genotype and allele frequencies of downstream effector cytokine genes (IL-17 and IFN-γ) in UA and control groups

Gene / Locus	**Genotype / Allele**	**Control n (%)**	**UA n (%)**	**OR (95% CI)a**	***p* value**
IL-17 rs2275913	GG	142 (50.7)	50 (31.3)	1.00 REF	–
	GA	113 (40.4)	86 (53.8)	**2.16 (1.41–3.31)**	< 0.001
	AA	25 (8.9)	24 (15.0)	**2.73 (1.43–5.20)**	0.003
	G allele	397 (70.9)	186 (58.1)	1.00 REF	–
	A allele	163 (29.1)	134 (41.9)	**1.75 (1.32–2.34)**	< 0.001
IFN-γ +874 A/T	AA	154 (55.0)	81 (50.6)	1.00 REF	–
	AT	112 (40.0)	60 (37.5)	1.02 (0.67–1.54)	0.985
	TT	14 (5.0)	19 (11.9)	**2.58 (1.23–5.41)**	0.017
	A allele	420 (75.0)	222 (69.4)	1.00 REF	–
	T allele	140 (25.0)	98 (30.6)	1.32 (0.98–1.80)	0.084

Abbreviations: UA, unstable angina; OR, odds ratio; CI, confidence interval.

[a]Adjusted by logistic regression for age, sex, BMI, and smoking.

Table 6. Association of cytokine gene polymorphisms with UA under different genetic models

Gene / Locus	Genetic model	Genotype comparison	OR (95% CI)a	*p* value
IL-1β −511 C/T	Codominant	CC vs CT vs TT	1.00 / 5.02 (2.21–11.49) / 6.39 (2.94–13.87)	< 0.001
	Dominant	(CT + TT) vs CC	5.84 (2.72–12.56)	< 0.001
	Recessive	TT vs (CC + CT)	2.01 (1.12–3.61)	0.021
IL-6 −1363 G/T	Codominant	GG vs GT vs TT	1.00 / 0.52 (0.34–0.80) / 0.60 (0.34–1.06)	0.006
	Dominant	(GT + TT) vs GG	0.54 (0.37–0.79)	0.002
	Recessive	TT vs (GG + GT)	0.68 (0.39–1.19)	0.172
IL-10 −592 C/A	Codominant	CC vs CA vs AA	1.00 / 2.42 (1.43–4.08) / 2.28 (1.31–3.99)	< 0.001
	Dominant	(CA + AA) vs CC	2.36 (1.45–3.84)	< 0.001
	Recessive	AA vs (CC + CA)	1.29 (0.82–2.04)	0.265
IL-10 −1082 G/A	Codominant	GG vs GA vs AA	1.00 / 2.45 (1.49–4.02) / 2.55 (1.47–4.43)	< 0.001
	Dominant	(GA + AA) vs GG	2.49 (1.59–3.89)	< 0.001
	Recessive	AA vs (GG + GA)	1.16 (0.74–1.84)	0.516
IL-17 rs2275913	Codominant	GG vs GA vs AA	1.00 / 2.16 (1.41–3.31) / 2.73 (1.43–5.20)	< 0.001
	Dominant	(GA + AA) vs GG	2.26 (1.52–3.36)	< 0.001
	Recessive	AA vs (GG + GA)	1.53 (0.88–2.65)	0.132
IFN-γ +874 A/T	Codominant	AA vs AT vs TT	1.00 / 1.02 (0.67–1.54) / 2.58 (1.23–5.41)	0.012
	Dominant	(AT + TT) vs AA	1.27 (0.85–1.90)	0.244
	Recessive	TT vs (AA + AT)	2.43 (1.17–5.05)	0.018
TNF-α −308 G/A	Codominant	GG vs GA vs AA	1.00 / 2.01 (1.23–3.27) / 2.56 (1.50–4.37)	< 0.001
	Dominant	(GA + AA) vs GG	2.21 (1.40–3.48)	< 0.001
	Recessive	AA vs (GG + GA)	1.50 (0.93–2.42)	0.095

Abbreviations: UA, unstable angina; OR, odds ratio; CI, confidence interval.

[a]Adjusted by logistic regression for age, sex, BMI, and smoking.

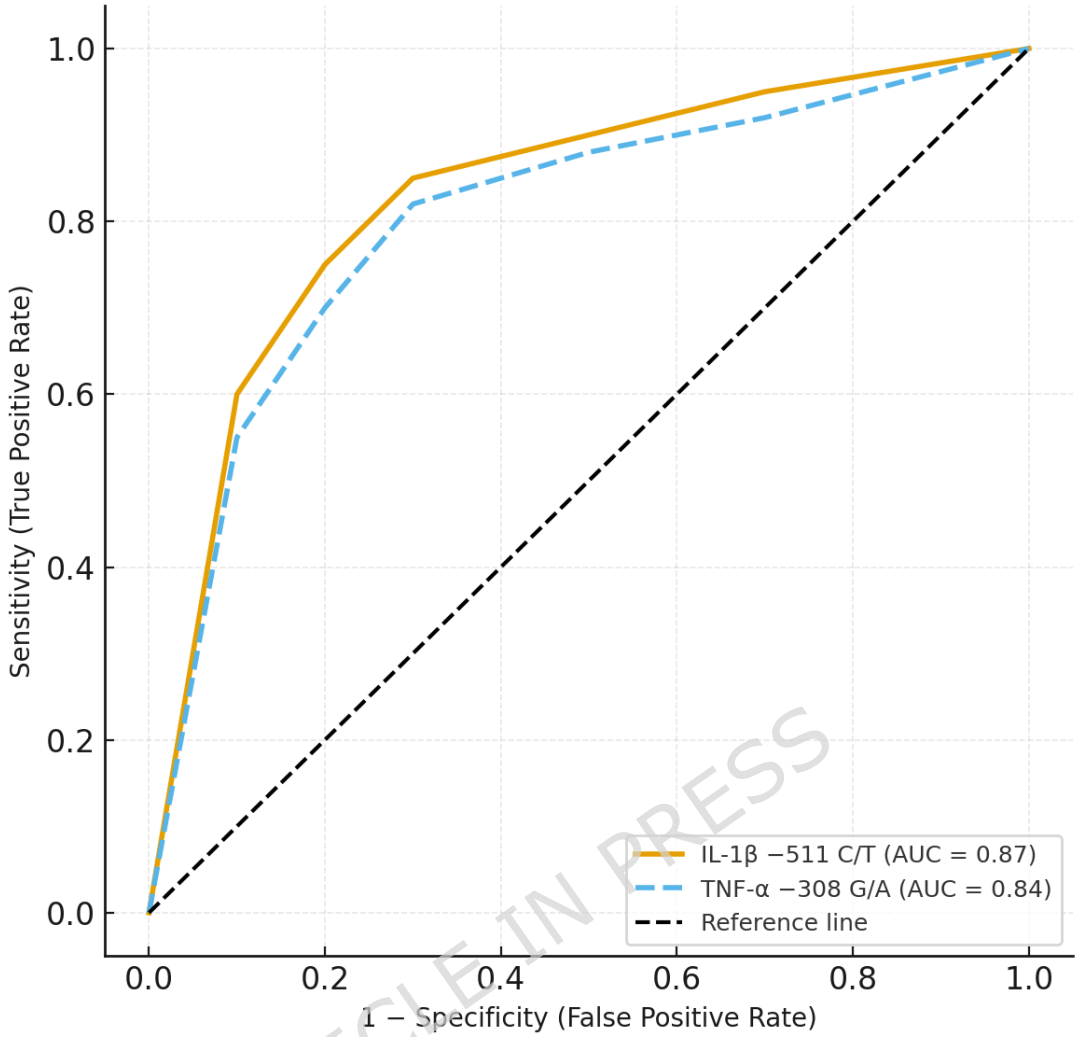

Figure 2. ROC curves for major cytokine gene polymorphisms in UA prediction.

Receiver operating characteristic (ROC) curves were generated using genotype (coded under the best-fitting genetic model) as the predictor and case status (UA vs. controls) as the endpoint. The IL-1β −511 C/T and TNF-α −308 G/A polymorphisms yielded areas under the curve (AUC = 0.87 and 0.84, respectively), indicating high sensitivity and specificity (>80%) for discriminating UA cases from controls.

3.4 Association between cytokine levels and genotypes

Significant correlations were observed between cytokine serum concentrations and corresponding genotypes. For instance, carriers

of the IL-1β −511 T allele exhibited higher IL-1β levels (9.2 ± 2.8 pg/mL) than CC carriers (6.1 ± 2.3 pg/mL, $p < 0.01$). Similarly, TNF-α −308 A allele carriers showed increased serum TNF-α (24.6 ± 5.8 pg/mL vs. 18.2 ± 4.9 pg/mL, $p < 0.01$). These genotype-phenotype associations support a functional impact of cytokine gene polymorphisms on inflammatory activation in UA. (Table 7.)

Table 7. Association between cytokine gene polymorphisms and corresponding serum cytokine levels in UA patients

Gene / Locus	Genotype / Allele	Serum cytokine concentration (pg/mL, mean ± SD)	*p* value
IL-1β −511 C/T	CC	6.1 ± 2.3	—
	CT + TT (T allele carriers)	9.2 ± 2.8	< 0.01
TNF-α −308 G/A	GG	18.2 ± 4.9	—
	GA + AA (A allele carriers)	24.6 ± 5.8	< 0.01
IL-6 −1363 G/T	GG	12.4 ± 3.9	—
	GT + TT (T allele carriers)	16.5 ± 4.4	0.02
IL-10 −1082 G/A	GG	7.1 ± 2.1	—
	GA + AA (A allele carriers)	9.5 ± 2.5	0.01
IL-17 rs2275913	GG	10.8 ± 3.2	—
	GA + AA (A allele carriers)	14.6 ± 3.7	< 0.01
IFN-γ +874 A/T	AA	13.2 ± 4.6	—
	AT + TT (T allele carriers)	17.4 ± 5.1	0.01

Abbreviations: UA, unstable angina; SD, standard deviation. Comparisons were performed using independent-sample t tests or Mann–Whitney U tests as appropriate.

Discussion

Inflammation plays a central role in the pathogenesis of atherosclerosis and its acute complications. UA represents a critical stage in CAD progression, characterized by plaque rupture and thrombosis driven by intense inflammatory activation [11–13]. Previous studies have established several key aspects of this process: complement activation in ruptured coronary plaques [11], inflammatory cell infiltration as a determinant of plaque vulnerability [12], and the prognostic value of circulating markers such as MPO and CRP for predicting adverse cardiovascular outcomes [14,15]. Additionally, IL-6 levels have shown correlation with both plaque burden and CAD risk [16,17], collectively underscoring the multifaceted involvement of inflammatory mechanisms in CAD pathogenesis.

Our findings substantially extend these established observations. We demonstrated significantly elevated serum levels of multiple inflammatory cytokines—including IL-1β, IL-6, IL-10, IL-17, IFN-γ, and TNF-α—in UA patients compared to healthy controls. Although IL-10 is classically considered anti-inflammatory, its marked elevation (3.2-fold) may reflect a compensatory response to heightened proinflammatory activity. Inflammatory stimuli such as TNF-α and IL-6 can induce IL-10 secretion from monocytes/macrophages in an attempt to downregulate excessive immune activation and limit tissue damage. More importantly, we identified significant associations between UA susceptibility and

specific polymorphisms in cytokine genes: IL-1β −511 C/T, TNF-α −308 G/A, IL-6 −1363 G/T, IL-10 −1082 G/A, IL-10 −592 C/A, IL-17 rs2275913, and IFN-γ +874 A/T. These genetic findings align with previous reports. The association between the TNF-α −308 A allele and increased angina pectoris risk has been documented [18], while IL-6 promoter polymorphisms have been linked to CAD in Han Chinese populations [16]. Similarly, IL-10 variants are known to influence anti-inflammatory regulation [19-21]. Notably, for the most significant loci in our study (IL-1β −511 C/T and TNF-α −308 G/A), both sensitivity and specificity exceeded 80% with statistical power >0.9, supporting their potential discriminative capacity for UA risk assessment.

A key mechanistic insight from our study is the observed genotype-phenotype correlation: carriers of the IL-1β −511 T and TNF-α −308 A alleles exhibited significantly higher corresponding cytokine levels. This suggests these polymorphisms may enhance transcriptional activity, thereby contributing to inflammatory cascades within coronary plaques. Correlation analysis revealed moderate positive associations between risk allele carriage and cytokine levels (e.g., $r = 0.43$ for IL-1β −511 T and IL-1β levels; $r = 0.39$ for TNF-α −308 A and TNF-α levels; both $p < 0.01$), reinforcing the functional significance of these variants. Similar functional relationships have been reported for IL-10 promoter polymorphisms that modulate cytokine secretion [19-21], reinforcing the biological plausibility that these variants actively contribute to plaque instability. Notably, the IL-1β −511 T allele is located within a putative NF-κB binding region, and its presence has been associated with enhanced promoter activity. Similarly, the TNF-α −308 A allele is known to disrupt transcriptional repressors, leading to higher gene expression. These variants may also affect

promoter methylation dynamics, as seen in previous epigenetic studies of atherosclerosis-related genes. TLR2 gene variants, for instance, influence susceptibility to both infectious and inflammatory diseases [22,23], highlighting the broader genetic landscape of immune regulation relevant to CAD.

While our study focused specifically on inflammatory cytokines, we acknowledge that other genetic pathways—such as oxidative stress and cell death—may contribute to vascular inflammation. However, their discussion is beyond the scope of the current analysis and warrants separate investigation in future studies integrating multi-pathway approaches [24-31]. Similarly, IL-18 gene variants have been linked to both cancer risk and vascular inflammation [32,22], emphasizing shared inflammatory mechanisms across different pathologies. These convergent findings support the concept that coronary inflammation results from complex gene-gene and gene-environment interactions, rather than isolated effects of single pathways.

The allele frequencies of the significant polymorphisms observed in our study (e.g., T allele frequency of 63.6% for IL-1β −511 C/T and A allele frequency of 43.2% for TNF-α −308 G/A in controls) were largely consistent with those previously reported in Chinese populations based on dbSNP and HapMap data. This concordance supports the representativeness of our sample and reduces the likelihood of population stratification bias.

From a translational perspective, while polymorphisms such as IL-1β −511 C/T and TNF-α −308 G/A demonstrated robust diagnostic characteristics (AUC ≥ 0.84) in our cohort, their interpretation as clinical biomarkers requires caution. Genetic variants may serve as useful susceptibility markers, but their predictive utility for individual risk stratification needs validation in

larger, multi-center, and multi-ethnic cohorts. Nevertheless, the consistent allele-cytokine concentration associations suggest a plausible mechanistic pathway linking genetic regulation to systemic inflammation and subsequent plaque instability, potentially informing future precision medicine approaches in CAD management.

Methodologically, our analytical framework—incorporating logistic regression adjustment, HWE testing, and model-based genetic comparisons—aligns with established standards for genetic association studies [24-26]. The observed HWE disequilibrium at several loci, particularly in controls, necessitated cautious interpretation of genotype distributions and prioritized allelic analyses. Deviations from HWE may arise from several causes, including genotyping errors, undetected population substructure, or selection bias during recruitment. However, repeat genotyping of 10% of samples showed >99% concordance, arguing against systematic laboratory error. Moreover, the overall allele frequencies remained within expected ranges, suggesting minimal impact on association results. The comprehensive evaluation of diagnostic performance through sensitivity, specificity, and power analysis strengthens our findings and adheres to recommended practices for biomarker validation.

Several limitations warrant consideration. The single-center, hospital-based design and moderate sample size may affect generalizability and precision for rare genotypes. The absence of functional assays limits direct mechanistic validation of the polymorphisms' effects. While key confounders were controlled, residual influences from medications, diet, or environmental exposures cannot be fully excluded. Although most UA patients received standard anti-ischemic therapy (e.g., aspirin, statins)

before blood sampling, we attempted to collect samples prior to full-dose maintenance treatment when feasible. Nonetheless, some agents may have modulated cytokine expression, and future studies should stratify analyses by treatment status to better isolate inflammatory signals. Future research integrating multi-omics approaches—GWAS, transcriptomics, epigenomics—could provide deeper insights, as demonstrated by recent studies combining miRNA profiling [34], gene expression signatures [35], and extracellular matrix gene variants [36].

Conclusion

In summary, our study demonstrates that key cytokine gene polymorphisms—particularly IL-1β −511 C/T and TNF-α −308 G/A—are functionally associated with elevated cytokine levels and increased UA risk. These findings strengthen the genetic and functional link between inflammatory dysregulation and plaque destabilization. While requiring larger-scale validation, this work provides a framework for integrating genetic and molecular data to advance our understanding of inflammation-driven coronary syndromes.

Ethics Approval Statement

This study was conducted with approval from the Ethics Committee of Deyang People's Hospital. This study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants.

Consent for publication

Written informed consent was obtained from all participants.

Data Sharing Statement

De-identified datasets, including aggregated genotype frequencies and anonymized cytokine concentration data, are available from the corresponding author upon reasonable request for academic purposes, in compliance with institutional ethics approval.

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Competing interests

The authors declare that they have no competing interests.

Author Contributions

Qiulan Yang and Runfeng Zhang conceived study design and content concept; Jie Lou and QianZhen Huang performed the data collection, extraction and analyzed the data; Jie Lou and QianZhen Huang were responsible for literature search; Qiulan Yang and Runfeng Zhang interpreted and reviewed the data and drafts.

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Figure legends

Figure 1. Serum cytokine expression levels in unstable angina (UA) and control groups.

Serum concentrations of six key inflammatory cytokines—interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), and interleukin-17 (IL-17)—were measured by enzyme-linked immunosorbent assay (ELISA) in patients with unstable angina (UA, n = 160) and healthy controls (n = 280). Data are presented as mean ± standard deviation (SD). All cytokines were significantly upregulated in the UA group compared with controls ($p < 0.01$,

unpaired t-test). The figure illustrates the pro-inflammatory activation pattern characteristic of UA.

Figure 2. ROC curves for major cytokine gene polymorphisms in UA prediction.

Receiver operating characteristic (ROC) curves were generated using genotype (coded under the best-fitting genetic model) as the predictor and case status (UA vs. controls) as the endpoint. The IL-1β −511 C/T and TNF-α −308 G/A polymorphisms yielded areas under the curve (AUC = 0.87 and 0.84, respectively), indicating high sensitivity and specificity (>80%) for discriminating UA cases from controls.

Table 1 Participates characteristics of UA group and control group

characteristics	UA group (N=160)	control group (N=280)	*p*
Age	68.8±7.1	67.6±6.8	0.08
Men	115(71.9%)	192(68.6%)	0.47
BMI	24.8±1.8	24.6±1.8	0.26
Smokers	44(27.5%)	80(28.5%)	0.33
Drinking history	33(20.6%)	55(19.6%)	0.73
diabetes mellitus	32(20.0%)	52(18.6%)	0.83
hypertension	34(21.3%)	57(20.4%)	0.70
TG	1.7±0.5	1.7±0.4	1.00
TC	4.8±0.9	4.7±0.8	0.23
LDL	3.3±0.8	3.2±0.7	0.17
HDL	1.9±0.6	2.0±0.7	0.13

UA, unstable angina; BMI, body mass index; TG, triglyceride; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high density lipoprotein.

Table 2 IL-1B genotype and allele frequency for UA group and control group

IL-1B loci	control group (N=280)		UA group (N=160)		OR(95%CI) [a]	P[a]
	n	Percentage (%)	n	Percentage (%)		
-511 C/T						
CC	66	23.6	8	3.7	1.00[REF]	
TC	72	25.7	44	27.5	**5.02(2.21-11.49)**	**<0.001**
TT	142	50.7	110	68.8	**6.39(2.94-13.87)**	**<0.001**
C	204	36.4	60	18.8	1.00[REF]	
T	356	63.6	264	81.2	**2.52(1.81-3.50)**	**<0.001**
+3954 C/T						
CC	149	53.2	82	51.3	1.00[REF]	
TC	112	40.0	66	41.3	1.07(0.71-1.61)	0.821
TT	19	6.8	12	7.5	1.15(0.53-2.48)	0.880
C	410	73.2	230	71.9	1.00[REF]	
T	150	26.8	90	28.1	1.07(0.79-1.45)	0.725
-31 C/T						
CC	155	55.4	91	56.9	1.00[REF]	
TC	112	40.0	60	37.5	1.91(0.61-1.37)	0.735
TT	13	4.6	9	5.6	1.18(0.48-2.	0.893

					87)	
C	422	75.4	242	75.6	1.00[REF]	
T	138	24.6	78	24.4	0.99(0.72-1.36)	0.994

UA, unstable angina; OR, odds ratio; CI, confidential index; [a] Adjusted for by logistic regression model.

Table 4 IL-10 genotype and allele frequency for UA group and control group

IL-10 loci	control group (N=280)		UA group (N=160)		OR(95%CI)[a]	P[a]
	n	Percentage (%)	n	Percentage (%)		
-592 C/A						
CC	88	31.4	26	16.3	1.00[REF]	
CA	112	40.0	80	50.0	2.42(1.43-4.08)	**<0.001**
AA	80	28.6	54	33.7	2.28(1.31-3.99)	**0.003**
C	288	51.4	132	41.3	1.00[REF]	
A	272	48.6	188	58.7	1.51(1.14-1.99)	**0.004**
-1082 G/A						
GG	102	36.4	30	18.8	1.00[REF]	
GA	114	40.7	82	51.2	2.45(1.49-4.02)	**<0.001**
AA	64	22.9	48	30.0	2.55(1.47-4.43)	**<0.001**
G	318	56.8	142	44.4	1.00[REF]	
A	242	43.2	178	55.6	1.65(1.25-2.17)	**<0.001**
-819 T/C						
TT	160	57.1	102	63.8	1.00[REF]	
TC	90	32.2	44	27.5	0.77(0.49-1.19)	0.234

CC	30	10.7	14	8.7	0.73(0.37-1.45)	0.368
T	410	73.2	248	77.5	1.00[REF]	
C	150	26.8	72	22.5	0.79(0.57-1.10)	0.159

UA, unstable angina; OR, odds ratio; CI, confidential index; [a] Adjusted for by logistic regression model.

Table 3 IL-6 genotype and allele frequency for UA group and control group

IL-6 loci	control group (N=280)		UA group (N=160)		OR(95%CI) [a]	P[a]
	n	Percentage (%)	n	Percentage (%)		
-174 G/C						
GG	138	49.3	88	55.0	1.00[REF]	
GC	92	32.9	48	30.0	0.82(0.53-1.27)	0.433
CC	50	17.8	24	15.0	0.75(0.43-1.31)	0.387
G	368	65.7	224	70.0	1.00[REF]	
C	192	34.3	96	30.0	0.82(0.61-1.10)	0.219
-1363 G/T						
GG	115	41.1	90	56.2	1.00[REF]	
GT	118	42.2	48	30.0	**0.52(0.34-0.80)**	**0.004**
TT	47	16.8	22	13.8	0.60(0.34-1.06)	0.106
G	348	62.1	228	71.3	1.00[REF]	
T	212	37.9	92	28.7	**0.66(0.49-0.89)**	**0.007**

UA, unstable angina; OR, odds ratio; CI, confidential index; [a] Adjusted for by logistic regression model.

Table 6 IFN-γ genotype and allele frequency for UA group and control group

IFN-γ loci	control group (N=280)		UA group (N=160)		OR(95%CI) [a]	P[a]
	n	Percentage (%)	n	Percentage (%)		
+874 A/T						
AA	154	55.0	81	50.6	1.00[REF]	
AT	112	40.0	60	37.5	1.02(0.67-1.54)	0.985
TT	14	5.0	19	11.9	**2.58(1.23-5.41)**	**0.017**
A	420	75.0	222	69.4	1.00[REF]	
T	140	25.0	98	30.6	1.32(0.98-1.80)	0.084
+2108 A/G						
AA	141	50.4	78	48.7	1.00[REF]	
AG	112	40.0	66	41.3	1.07(0.71-1.61)	0.844
GG	27	9.6	16	10.0	1.07(0.54-2.11)	0.980
A	394	70.4	222	69.4	1.00[REF]	
G	166	29.6	98	30.6	1.05(0.78-1.41)	0.819

UA, unstable angina; OR, odds ratio; CI, confidential index; [a] Adjusted for by logistic regression model.

Table 5 IL-17 genotype and allele frequency for UA group and control group

IL-17 loci	control group (N=280)		UA group (N=160)		OR(95%CI) [a]	P[a]
	n	Percentage (%)	n	Percentage (%)		
rs2275913						
GG	142	50.7	50	31.3	1.00[REF]	
GA	113	40.4	86	53.8	**2.16(1.41-3.31)**	**<0.001**
AA	25	8.9	24	15.0	**2.73(1.43-5.20)**	**0.003**
G	397	70.9	186	58.1	1.00[REF]	
A	163	29.1	134	41.9	**1.75(1.32-2.34)**	**<0.001**
rs763780						
TT	149	53.2	80	50.0	1.00[REF]	
TC	112	40.0	60	37.5	1.00(0.66-1.51)	0.924
CC	19	6.8	20	12.5	1.96(0.99-3.89)	0.076
T	410	73.2	220	68.8	1.00[REF]	
C	150	26.8	100	31.2	1.24(0.92-1.68)	0.182

UA, unstable angina; OR, odds ratio; CI, confidential index; [a] Adjusted for by logistic regression model.

Table 7 TNF-α genotype and allele frequency for UA group and control group

TNF-α loci	control group (N=280)		UA group (N=160)		OR(95%CI)[a]	P[a]
	n	Percentage (%)	n	Percentage (%)		
-308 G/A						
GG	102	36.4	33	20.6	1.00[REF]	
GA	114	40.7	74	46.3	2.01(1.23-3.27)	**0.007**
AA	64	22.9	53	33.1	2.56(1.50-4.37)	**<0.001**
G	318	56.8	140	43.8	1.00[REF]	
A	242	43.2	180	56.2	1.69(1.28-2.23)	**<0.001**
-376 G/A						
GG	139	49.6	78	48.7	1.00[REF]	
GA	111	39.6	66	41.3	1.06(0.70-1.60)	0.865
AA	30	10.8	16	10.0	0.95(0.49-2.85)	0.984
G	389	69.5	222	69.4	1.00[REF]	
A	171	30.5	98	30.6	1.00(0.75-1.35)	0.961

UA, unstable angina; OR, odds ratio; CI, confidential index; [a] Adjusted for by logistic regression model.