Analysis of the genetic association between IL27 variants and coronary artery disease in a Chinese Han population

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Interleukin-27 (IL-27) is an important cytokine in inflammatory diseases, including coronary artery disease (CAD). To explore the precise role of IL-27 in CAD, we investigated the genetic association between IL27 and CAD in the GeneID Chinese Han population. A two-stage case control association analysis was performed for 3075 CAD cases and 2802 controls. Logistic regression analysis was used to adjust the traditional risk factors for CAD. Results showed that a promoter variant, rs153109, tended to be marginally associated with CAD in the discovery population ($P_{adj} = 0.028$, OR = 1.27, 95%CI: 1.03–1.58). However, this association was not replicated in the validation stage ($P_{adj} = 0.559$, OR = 1.04, 95%CI: 0.90–1.21). In addition, when we classified the combined population into two subgroups according to the age at disease onset or disease state, we again obtained no significant associations. Finally, we estimated the severity of coronary stenosis using the Gensini Scoring system and determined that the rs153109 genotypes were still not associated with the Gensini scores of the CAD patients. In conclusion, our study failed to find an association between common variants in the functional region of IL27 and CAD in a Chinese Han population, which indicated that IL-27 might only be an inflammatory marker during the development of CAD.

Coronary artery disease (CAD), the leading cause of death and infirmity worldwide, is a complex disease caused by multiple genetic and environmental factors, as well as the interactions between them. Although genome-wide association analysis studies have identified more than 50 risk loci for CAD in recent years\textsuperscript{1–3}, the heritability of CAD is still incompletely understood\textsuperscript{4,5}. Therefore, more studies are urgently needed to identify the genetic factors to fully explain the molecular genetic mechanisms of CAD and provide valuable suggestions for the prevention and treatment of CAD.

Atherosclerosis is the basis for CAD pathogenesis. Inflammation plays an important role and is involved in the initiation and progression of atherosclerosis\textsuperscript{6}. Recent studies show that genetic variations in inflammatory cytokines are involved in the process, including IL6, IL16, IL17A, CRP, and IL33\textsuperscript{2,7–10}. IL-27 is a new member of the IL-6/IL-12 family and comprises an Epstein-Barr virus-induced molecule 3 (EBI3) subunit joined with the...
association between the three SNPs (rs181206, rs17855750 and rs38433) and CAD (OR for age, sex, smoking, BMI, hypertension, diabetes mellitus, and the lipid concentrations (rs153109C, discovery stage, the allelic frequency of rs153109 tended to be marginally associated with CAD after adjusting to explain the exact function of IL-27 in the development of CAD.

p28 subunit11. IL-27 plays an important role in the innate and adaptive immune systems. Variants in the IL27 gene contribute to the risk of multiple inflammatory immune diseases, such as inflammatory bowel disease, rheumatoid arthritis, asthma, and chronic obstructive pulmonary disease12–16. However, the role of the IL-27 in atherosclerosis is uncertain. In 2011, Jafarzadeh et al. reported that the serum level of IL-27 was elevated in patients with ischemic heart diseases27. Later, Jin et al. found that elevated level of IL-27 in the circulation of CAD patients was significantly associated with the level of ox-LDL and the severity of coronary artery stenosis, as estimated by the Gensini Scoring system28. This evidence indicates that IL-27 might play a causal role in the development of CAD. However, in contrast, some studies found that IL-27 and its receptor could inhibit the activity of macrophages and may retard the process of atherosclerosis19,20. These controversial findings regarding the relationship between IL-27 and CAD prompted us to investigate the genetic role of IL27 and CAD.

Table 1. Clinical characteristics of the studied GeneID Chinese Han population. The data are presented as the mean ± standard deviation or a percentage; CAD, coronary artery disease; Tch, total cholesterol; TG, triglyceride; LDL-c, low-density lipoprotein cholesterol.

| Characteristics | GeneID-discovery |  | GeneID-validation |  | GeneID-combined |  |
|-----------------|------------------|---|-------------------|---|-----------------|---|
| Subject numbers | CAD              |  | Control           |  |               |  |
| Age (years)     | 63.0 ± 11.2      |  | 62.0 ± 11.6       |  | 62.3 ± 11.6    |  |
| Gender (male %) | 70.1             |  | 59.2              |  | 66.3 ± 11.6    |  |
| Smoking (%)     | 44.7             |  | 16.2              |  | 3.08 ± 11.7    |  |
| BMI (kg/m²)     | 24.2 ± 1.59      |  | 23.7 ± 1.33       |  | 21.8 ± 1.32    |  |
| Hypertension (%)| 66.3             |  | 15.7              |  | 13.5 ± 10.3    |  |
| DM (%)          | 34.7             |  | 3.40              |  | 3.91 ± 7.8     |  |
| TG (mmol/l)     | 1.69 ± 1.09      |  | 1.51 ± 1.20       |  | 1.28 ± 10.4    |  |
| LDL-c (mmol/l)  | 2.99 ± 1.03      |  | 2.75 ± 0.63       |  | 1.85 ± 10.2    |  |

Clinical characteristics of the population. Table 1 illustrates the detailed clinical features of the two populations enrolled in this study. As expected, the age, BMI, TG, Tch and LDL-c levels were significantly higher in the CAD patients than in the controls. The male percent and the prevalence of smoking, hypertension and diabetes mellitus were also significantly increased in the CAD patients compared with the controls.

With an effect size of 1.2 and a minor allele frequency based on the Hap Map CHB and JPT datasets, the statistical power for all of the studied variants was greater than 50% in the discovery populations and greater than 95% in the validation populations. The combined sample size also provided a statistical power of greater than 100% for rs153109.

Association analysis of four tag SNPs in the GeneID Chinese Han population. All selected variants passed the Hardy-Weinberg equilibrium test, with P values greater than 0.001 in the control subjects. In the discovery stage, the allelic frequency of rs153109 tended to be marginally associated with CAD after adjusting for age, sex, smoking, BMI, hypertension, diabetes mellitus, and the lipid concentrations (rs153109C, Padj = 0.028, OR = 1.27, 95%CI: 1.03–1.58). The other three variants were not associated with CAD (rs181206G, Padj = 0.719, OR = 0.93, 95%CI: 0.64–1.36; rs17855750C, Padj = 0.229, OR = 1.22, 95%CI: 0.88–1.69; rs348337, Padj = 0.247, OR = 1.17, 95%CI: 0.90–1.54) (Table 2). Using the genotypic association analysis, there was no significant association between the three SNPs (rs181206, rs17855750 and rs38433) and CAD (Padj > 0.05) (Table 3). Thus, no further analysis was performed on these variants. In the validation cohort, the association between rs153109 and CAD was not further verified after adjusting for covariates (rs153109, Padj = 0.559, OR = 1.04, 95%CI: 0.90–1.21). After combining the two cohorts, we performed a combined association analysis (3075 CAD cases vs 2802 controls) between rs153109 and CAD, which also showed that there was no significant association (rs153109, Padj = 0.164, OR = 1.08, 95%CI: 0.97–1.21). Moreover, no significant genotypic association was identified in different cohorts (Tables 2 and 3).

Stratified analysis for the association between rs153109 and CAD by the age at disease onset and disease state in the GeneID-combined Population. An additional statistical analysis was performed by dividing the total CAD patients into two subgroups by the age at disease onset: one is the early-onset CAD subgroup with 847 individuals, and the other is the late-onset CAD subgroup with 2157 subjects. The early-onset CAD subgroup was defined as having an age of CAD onset of less than 55 years for male patients and less than 65 years for female patients21. Using the allelic and genotypic association analyses, there were no significant associations between rs153109 and CAD in the early-onset and late-onset subgroup populations after adjusting for the risk factors (Padj > 0.05 for both the allelic and genotypic associations) (Tables 4 and 5). The Breslow–Day test for heterogeneity was not significant (P = 0.844), suggesting the homogeneity of different disease onset age groups.
In addition, we divided the CAD population into two subgroups by the disease state: one is the anatomical-CAD group, consisting of approximately 1205 CAD subjects with severe coronary stenosis, and the other is the clinical-CAD group, which includes 1799 CAD subjects with myocardial infarction or revascularization\textsuperscript{2}. The results showed that rs153109 was not significantly associated with CAD in both subgroups after adjustment.

### Table 2. Allelic association analysis between IL27 and CAD in the GeneID Chinese Han population.

| Population          | Model | N    | MAF     | \(P_{\text{obs}}\) | \(P_{\text{adj}}\) | OR (95%CI) |
|---------------------|-------|------|---------|---------------------|---------------------|------------|
| GeneID-discovery    |       |      |         |                     |                     |            |
| (1245/1077)         | ADD   | 7/191/1047 | 11/166/900 | 0.456               | 0.720               | 0.93 (0.64–1.36) |
|                     | DOM   | 198/1047   | 177/900   | 0.795               | 0.919               | 0.98 (0.66–1.47) |
|                     | REC   | 7/1238     | 11/1066   | 0.210               | 0.218               | 0.33 (0.06–1.94) |
| rs181206\textsuperscript{G} |       |      |         |                     |                     |            |
| rs17855750\textsuperscript{C} |       |      |         |                     |                     |            |
| rs34833\textsuperscript{T} |       |      |         |                     |                     |            |
| rs153109\textsuperscript{C} |       |      |         |                     |                     |            |
| GeneID-validation   | ADD   | 36/389/758 | 32/287/656 | 0.265               | 0.225               | 1.19 (0.90–1.59) |
| (1830/1725)         | DOM   | 425/758    | 319/656   | 0.142               | 0.165               | 1.25 (0.91–1.72) |
|                     | REC   | 36/1147    | 32/943    | 0.754               | 0.902               | 0.94 (0.35–2.51) |
| rs153109\textsuperscript{C} |       |      |         |                     |                     |            |
| GeneID-combined     | ADD   | 180/607/1387 | 141/47/367 | 0.103               | 0.024               | 1.29 (1.04–1.61) |
| (3075/2802)         | DOM   | 787/387    | 615/367   | 0.033               | 0.055               | 1.36 (1.00–1.87) |
|                     | REC   | 180/994    | 141/841   | 0.564               | 0.085               | 1.45 (0.95–2.20) |
| rs153109\textsuperscript{C} |       |      |         |                     |                     |            |
| rs153109\textsuperscript{C} |       |      |         |                     |                     |            |

### Table 3. Genotypic association of the tag SNPs in IL27 with CAD in the GeneID Chinese Han population.

| Population          | Model | N    | MAF     | \(P_{\text{obs}}\) | \(P_{\text{adj}}\) | OR (95%CI) |
|---------------------|-------|------|---------|---------------------|---------------------|------------|
| GeneID-discovery    | ADD   | 7/191/1047 | 11/166/900 | 0.456               | 0.720               | 0.93 (0.64–1.36) |
| (1245/1077)         | DOM   | 198/1047   | 177/900   | 0.795               | 0.919               | 0.98 (0.66–1.47) |
|                     | REC   | 7/1238     | 11/1066   | 0.210               | 0.218               | 0.33 (0.06–1.94) |
| rs181206\textsuperscript{G} |       |      |         |                     |                     |            |
| rs17855750\textsuperscript{C} |       |      |         |                     |                     |            |
| rs34833\textsuperscript{T} |       |      |         |                     |                     |            |
| rs153109\textsuperscript{C} |       |      |         |                     |                     |            |
| GeneID-validation   | ADD   | 36/389/758 | 32/287/656 | 0.265               | 0.225               | 1.19 (0.90–1.59) |
| (1830/1725)         | DOM   | 425/758    | 319/656   | 0.142               | 0.165               | 1.25 (0.91–1.72) |
|                     | REC   | 36/1147    | 32/943    | 0.754               | 0.902               | 0.94 (0.35–2.51) |
| rs153109\textsuperscript{C} |       |      |         |                     |                     |            |
| GeneID-combined     | ADD   | 180/607/1387 | 141/47/367 | 0.103               | 0.024               | 1.29 (1.04–1.61) |
| (3075/2802)         | DOM   | 787/387    | 615/367   | 0.033               | 0.055               | 1.36 (1.00–1.87) |
|                     | REC   | 180/994    | 141/841   | 0.564               | 0.085               | 1.45 (0.95–2.20) |
| rs153109\textsuperscript{C} |       |      |         |                     |                     |            |

### Table 4. Allelic association analysis of rs153109 in the different subgroups of the GeneID-combined population.

| Population          | Model | N    | MAF     | \(P_{\text{obs}}\) | \(P_{\text{adj}}\) | OR (95%CI) |
|---------------------|-------|------|---------|---------------------|---------------------|------------|
| CAD-early-onset     | ADD   | 7/191/1047 | 11/166/900 | 0.456               | 0.720               | 0.93 (0.64–1.36) |
| rs153109\textsuperscript{C} |       |      |         |                     |                     |            |
| CAD-late-onset      | ADD   | 2157/1047  | 177/900   | 0.795               | 0.919               | 0.98 (0.66–1.47) |
| rs153109\textsuperscript{C} |       |      |         |                     |                     |            |
| CAD-anatomical      | ADD   | 1205/758   | 1070/655  | 0.157               | 0.813               | 1.03 (0.83–1.26) |
| rs153109\textsuperscript{C} |       |      |         |                     |                     |            |
| CAD-clinical        | ADD   | 1799/758   | 1685/655  | 0.014               | 0.297               | 1.09 (0.93–1.28) |
| rs153109\textsuperscript{C} |       |      |         |                     |                     |            |
adjusted by the covariates; OR, odds ratio after adjustment; the P value adjusted for the risk factors. The first and fourth quartiles of the LN [Gensini score] distribution were used to perform the case control association analysis. The first quartile was defined as the quartile with the lowest Gensini scores, and the fourth quartile was defined as the quartile with the highest Gensini scores. Pobs, observed P value; Padj, P value adjusted by the covariates; OR, odds ratio after adjustment. ADD, additive model, rs153109_CC/CT/TT; DOM, dominant model, rs153109_CC+CT/TT; REC, recessive model, rs153109_CC/CT+TT.

| Population (n, case/control) | SNP-allele | Model | Cases | Controls | Pobs | Padj | OR (95%CI) |
|------------------------------|------------|-------|-------|----------|------|-------|------------|
| CAD-early-onset (847/2707)   | rs153109C  | ADD   | 131/146/300 | 366/1319/1022 | 0.258 | 0.380 | 1.07 (0.93–1.23) |
|                             |            | DOM   | 547/300  | 1685/1022 | 0.220 | 0.634 | 1.05 (0.86–1.28) |
|                             |            | REC   | 131/716  | 366/2341 | 0.154 | 0.298 | 1.15 (0.88–1.51) |
| CAD-late-onset (2157/2707)   | rs153109C  | ADD   | 324/1093/740 | 366/1319/1022 | 0.034 | 0.320 | 1.08 (0.93–1.25) |
|                             |            | DOM   | 1417/740 | 1685/1022 | 0.013 | 0.562 | 1.06 (0.87–1.30) |
|                             |            | REC   | 324/1833 | 366/2341 | 0.136 | 0.262 | 1.18 (0.89–1.56) |
| CAD-anatomical (1205/2707)   | rs153109C  | ADD   | 182/584/439 | 366/1319/1022 | 0.388 | 0.617 | 1.04 (0.90–1.20) |
|                             |            | DOM   | 766/439  | 1685/1022 | 0.430 | 0.710 | 0.96 (0.78–1.18) |
|                             |            | REC   | 182/1023 | 366/2341 | 0.188 | 0.142 | 1.23 (0.93–1.63) |
| CAD-clinical (1799/2707)     | rs153109C  | ADD   | 273/925/601 | 366/1319/1022 | 0.010 | 0.149 | 1.10 (0.97–1.25) |
|                             |            | DOM   | 1198/601 | 1685/1022 | 0.003 | 0.153 | 1.14 (0.95–1.37) |
|                             |            | REC   | 273/1526 | 366/2341 | 0.119 | 0.411 | 1.11 (0.87–1.41) |

Table 5. Genotypic association analysis of rs153109 in the different subgroups of the GeneID-combined population. Pobs, observed P value; Padj, P value adjusted by the covariates; OR, odds ratio after adjustment. ADD, additive model, rs153109_CC/CT/TT; DOM, dominant model, rs153109_CC+CT/TT; REC, recessive model, rs153109_CC/CT+TT.

Table 6. Genotypic association analysis of rs153109 and the LN-transformed Gensini scores in 1488 CAD patients. The first and fourth quartiles of the LN [Gensini score] distribution were used to perform the case control association analysis. The first quartile was defined as the quartile with the lowest Gensini scores, and the fourth quartile was defined as the quartile with the highest Gensini scores. Pobs, observed P value; Padj, P value adjusted by the covariates; OR, odds ratio after adjustment; the Padj values and OR values were obtained using a multivariate logistic regression analysis.

| SNP-allele | Quantitative trait association | Case control association |
|------------|-------------------------------|--------------------------|
|            | RAF (n) | beta | SE  | r²  | Pobs | Padj | OR (95%CI) | 1st(245) | 4th(394) | Pobs | Padj | OR (95%CI) |
| rs153109C  |         | 0.002 | 0.014 | 0.008 | 0.587 | 0.277 | 0.391 | 0.386 | 0.854 | 0.646 | 0.94 (0.74–1.21) |

Table 5. Genotypic association analysis of rs153109 in the different subgroups of the GeneID-combined population. Pobs, observed P value; Padj, P value adjusted by the covariates; OR, odds ratio after adjustment. ADD, additive model, rs153109_CC/CT/TT; DOM, dominant model, rs153109_CC+CT/TT; REC, recessive model, rs153109_CC/CT+TT.

Table 6. Genotypic association analysis of rs153109 and the LN-transformed Gensini scores in 1488 CAD patients. The first and fourth quartiles of the LN [Gensini score] distribution were used to perform the case control association analysis. The first quartile was defined as the quartile with the lowest Gensini scores, and the fourth quartile was defined as the quartile with the highest Gensini scores. Pobs, observed P value; Padj, P value adjusted by the covariates; OR, odds ratio after adjustment; the Padj values and OR values were obtained using a multivariate logistic regression analysis.

Adjusting for the risk factors (Padj > 0.05 for the allelic and genotypic associations) (Tables 4 and 5). The Breslow–Day test for heterogeneity was not significant (P = 0.335), suggesting the homogeneity of different disease state groups.

Analysis of the association between rs153109 and the Gensini scores in the GeneID-CAD population. We further investigated the possible association of rs153109 in IL27 with the severity of CAD, as estimated by the Gensini Scoring system, in 1488 CAD cases who had received a coronary angiography examination. After loge-transformation, we performed a quantitative trait association analysis and a quartile case control association analysis between the genotypes of rs153109 and the Gensini scores. Unfortunately, rs153109 was not statistically correlated with the severity of atherosclerosis, as estimated by the Gensini scores (Table 6 and Fig. 1).

Discussion

In this study, we aimed to evaluate the contribution of polymorphisms in the IL27 gene to CAD susceptibility in Chinese Han subjects by employing a two-stage case control association analysis. Our study demonstrated that none of the variants from the functional region were different between the CAD cases and control subjects. IL-27, a heterologous dimer composed of EBI3 and p28, was shown to promote CD4+ T cell proliferation. IL-27R is the orphan receptor of IL-27 and is expressed in a variety of inflammatory immune cells such as monocytes, macrophages, dendritic cells, mast cells, NK cells, endothelial cells, and T/B lymphocytes. IL-27 could regulate the proliferation, differentiation and maturation of multiple immune cells and was involved in inflammatory immune diseases. Epidemiological studies have suggested that the serum IL-27 levels were elevated in patients with multiple sclerosis, psoriasis, Behcet’s disease, and visceral leishmaniasis but were attenuated in patients with rheumatoid arthritis and systemic lupus erythematosus disease. Some clinical experiments revealed that IL-27 could prolong survival of patients with glomerulonephritis and improve the joint pathology of patients with arthritis but aggravated airway inflammation in allergic asthma patients. Additionally, new studies also found that genetic polymorphisms in IL-27 were significantly associated with multiple inflammatory immune diseases. These results suggested that IL-27 might play important roles in the pathogenesis of inflammatory autoimmune diseases, but the molecular mechanisms are probably quite different.

From 2011 to 2013, several researchers were involved in uncovering the relationship between IL-27 and CAD and reported that IL-27 might play a dual role in promoting and inhibiting different stages of the development of CAD. The contradictory findings for the role of IL-27 in the development of CAD in previous studies might be explained by an unknown biological interaction between IL-27 and other known or unknown CAD risk factors. In our genetic research, we demonstrated that the allelic and genotypic frequencies were not associated with the
susceptibility to CAD in a Chinese Han population, even though the rs153109 variant tended to be marginally associated with CAD in the discovery stage. In addition, a subsequent analysis also found that the association between rs153109 and CAD was not significant in the different stratifications and stenosis severities. Recently, another study indicated that variants in \(\text{IL27}\) did not differ between T1D patients and the controls in a Brazilian population, even when these variants were analyzed together with the major HLA-DRB1 risk alleles41. Another study also found that a genetic variant at the \(\text{IL27}\) locus was associated with T1D, which had a strong cis-eQTL effect on CCDC101 instead of the \(\text{IL27}\) gene42. Interestingly, CAD and T1D are both metabolic diseases, and an imbalance in Th1/Th2 cell function and a disrupted oxidative stress response play important roles in the pathogenesis of these diseases. Based on the above evidence, we might conclude that the inflammatory cytokine IL-27 was only an inflammatory marker during the development of CAD.

There were some disadvantages in our study. First, the relatively small number of subjects in this study might lead to false negative association results. Second, the studied subjects were enrolled from different centers in China and might have different environmental and genetic risks. Third, although we selected tagged SNPs in the functional region of \(\text{IL27}\), more variants in the non-coding regions surrounding the gene or between exons might be missed. Finally, some control subjects might develop cardiovascular diseases in the future.

In conclusion, although \(\text{IL27}\) is an attractive candidate that may contribute to inflammatory immune diseases, our study failed to find an association between common variants in the functional region of \(\text{IL27}\) and CAD in a Chinese Han population. However, this finding needs to be confirmed in a larger sample and in different ethnic groups.

Methods

Study Population. A two-stage case control genetic association study was performed with a total of 5877 subjects (3075 CAD cases vs 2802 controls). All subjects were enrolled from the GeneID Chinese Han population, which is a large ongoing database that aims to study the genetic basis of cardiovascular diseases. In the first stage, 2322 subjects (1245 CAD cases and 1077 controls) from the GeneID-Central-China population were enrolled from Hubei province and served as the discovery cohort. In the second stage, 3555 subjects (1830 CAD cases and 1725 controls) from the GeneID-Northern-China population were enrolled from Shandong and Liaoning provinces and served as the validation cohort. The criterion for the enrollment as a CAD case was defined as a stenosis diameter of 70% in any of the main coronary arteries (left main, left anterior descending, left circumflex artery or right coronary artery) by coronary angiography, a coronary artery bypass graft, percutaneous coronary intervention, and/or myocardial infarction. Subjects who had experienced myocardial spasms or had a myocardial bridge, as identified by angiography, or had congenital heart disease, cardiomyopathy, heart valve disease, renal or hepatic disease, and autoimmune diseases were excluded from the study. The control subjects were selected from individuals whose major coronary artery displayed no more than 30% stenosis as confirmed by angiography and who had no history of CAD. The disease states of hypertension and diabetes mellitus were evaluated according to published guidelines43,44. The fasting total cholesterol (Tch), triglyceride (TG) and LDL cholesterol (LDL-c) concentrations were measured using standard methods45,46. Direct interviews and medical record reviews were performed to collect the subjects' clinical characteristics, such as age, gender, body mass index (BMI), and smoking history. This study was approved by the Medical Ethical Committee of Huazhong University of Science and Technology and complied with the ethical principles set forth by the Declaration of Helsinki. All participants provided the informed consent.

Gensini scores. The severity of coronary artery stenosis was estimated using the Gensini Scoring system46,47. For each segment, the score was rated as 1, 2, 4, 8, 16 and 32 for coronary stenosis of 0–25%, 26–50%, 51–75%, 76–90%, 91–99% and 100%, respectively. After multiplying the factor assigned to each segment with the vessel...
size and importance (ranging from 0.05 to 5.0), the Gensini index for 1488 cases undergoing conventional angio-
graphy was calculated by adding the total weights for each segment.

**Genetic Analysis.** The DNA samples were collected from the peripheral blood. The map position within the region of 15420bp was shown in the regional plot (Fig. 2). The tag SNPs were selected according to the following principles: (1) construction of a linkage disequilibrium (LD) map with the single nucleotide polymorphisms (SNPs) of \( \text{IL27} \) using Haplovew (v.4.2) and the HapMap CHB and JPT datasets (v.3, release 2), with thresholds of \( r^2 > 0.8 \) to reduce redundancy; (2) variants with a minor allele frequency of more than 0.05; (3) functional variants reported by previous studies; (4) variants located in the promoter region or exon region; (5) and the LD block was analyzed using the four-gamete rule. Finally, four tag SNPs (rs181206, rs17855750, rs34833 and rs153109) that completely covered the \( \text{IL27} \) gene were selected. rs181206 and rs17855750 are located in the exon region, which might affect the function of \( \text{IL27} \) protein. rs34833 and rs153109 are located in the predicted promoter region, which might regulate the expression of \( \text{IL27} \) (Fig. 2).

All of the subjects in this study were genotyped using a Rotor-Gene 6000 High-Resolution Melt (HRM) system (Corbett Life Science, Concorde, NSW, Australia). Genotyping was performed by PCR in a total reaction volume of 25 µl PCR, containing 0.7 µl of LC Green dye, 10 pmol of each primer, 30 ng of genomic DNA, 2.5 µl of 10 × PCR buffer with 1.5 mmol/L MgCl₂, 5 mmol deoxynucleotide triphosphates, and 1 unit of Taq polymerase. The genotyping results were verified by direct DNA sequencing analysis with 48 cases and 48 controls randomly selected from the studied subjects. The success rates for the different SNPs ranged from 92.9 to 100%.

**Statistical Analysis.** The Hardy-Weinberg disequilibrium test was performed on the controls (plink). The allelic and genotypic association analyses were performed using Pearson’s 2 × 2 and 2 × 3 contingency table chi-square tests; the odds ratio (OR) and 95% confidence interval (CI) were also calculated (SPSS, v.17.0). Age, gender, BMI, hypertension, diabetes mellitus, smoking history, Tch, TG, and LDL-c were studied and analyzed as covariates using multiple logistic regression analysis. Breslow-Day tests were used to assess the heterogeneity from different subgroups using SPSS (version 17.0). The statistical power analysis was performed with a free program that calculates the sample size and power (PS v.3.0.12). After log₂-transformation, the Gensini scores were analyzed using a linear regression analysis and a quartile case control association analysis.
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**Author Contributions**
Conceived and designed the experiments: X.C., X.T. and Q.K.W. Performed the experiments and analyzed the data: Q.F., S.F.N., S.H.L. and Y.H.L. Contributed reagents/materials/analysis tools: Q.F., H.S.Z., L.F.Z., F.W., T.T.T., N.X., C.Q.X., P.Y.W., T.X., J.J.X., Q.L.L., Q.X.L., J.Q., B.L., G.W., Y.X.W. and Y.Y. Wrote the paper: Q.F. All authors reviewed the manuscript.

**Additional Information**
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