Acute aortic dissection (AAD) is the most life-threatening macrovascular disorder with high mortality, characterized by intimal tear and surging of blood into the medial layer of the aorta [1, 2]. The etiology of AAD is complex and heterogeneous. In addition to some environmental risk factors, such as smoking, male, old age, hypertension, and dyslipidemia, genetic factors are also viewed to contribute to the pathogenesis of AAD [3, 4]. Although the responsible molecular and genetic determinants of AAD remain largely unidentified, it has been widely accepted that inflammation plays an essential role in the structural damage of the aortic wall and the development of AAD [2, 5, 6]. Increasing data indicated that elevated inflammatory cell infiltration and higher expression levels of inflammatory mediators, including C-reactive protein (CRP) and D-dimer, were detected in dissected aortic specimens and peripheral blood of AAD patients [7, 8].

### 1. Introduction

AAD is a macrovascular disorder characterized by intimal tear and blood flow from one aortic lumen into the other. This condition is associated with a high mortality rate, ranging from 8% to 43% [9]. Several risk factors contribute to the development of AAD, including smoking, male, old age, hypertension, and dyslipidemia [1, 2]. Genetic factors are also considered to play a role in the pathogenesis of AAD [3, 4]. The relationship between genetic variations and AAD susceptibility is an area of active research.

**Background.** Inflammation may be involved in the pathogenesis of acute aortic dissection (AAD). Toll-like receptor 4 (TLR4) is known to play a critical role in regulating the immune and inflammatory processes. To date, the relationship between genetic variation of TLR4 and AAD is far from clear. The purpose of our study was to illustrate the relevance of TLR4 polymorphisms with the susceptibility to AAD. Methods. A total of 222 AAD patients and 222 controls were enrolled in this study. Frequency distributions of TLR4 polymorphisms (rs10759932 in the promoter and rs11536889 in the 3′-untranslated region) were determined by the KASP method. Clinical parameters were acquired from subjects’ medical records, and serum TLR4 levels were collected from our previously published data. Results. We found that rs10759932 polymorphism was associated with a reduced risk of AAD in the overall population (CC vs. TT: OR = 0.393, 95%CI = 0.164-0.939, P = 0.036; recessive model: OR = 0.439, 95%CI = 0.196-0.984, P = 0.045) and subgroup analyses stratified by sex. The GC genotype and dominant model of rs11536889 conferred a significantly higher risk of AAD compared with GG genotype in female subjects (GC vs. GG: OR = 3.382, 95%CI = 1.051-10.885, P = 0.041; dominant model: OR = 3.043, 95%CI = 1.041-8.900, P = 0.042). In addition, a significant interaction between the rs11536889 recessive model and dyslipidemia was observed for an increased risk of AAD (Pinteraction = 0.038, OR = 15.229) after the adjustment for potential clinical covariates. We also used the false-positive report probability (FPRP) analysis to validate the significant results. Furthermore, rs11536889 polymorphism could affect the maximal aortic diameters of AAD (P = 0.037), while AAD patients carrying CC genotype of rs10759932 showed lower serum TLR4 levels than TT genotype carriers (P = 0.043). Conclusions. Our findings provide evidence for the association between TLR4 polymorphisms and AAD susceptibility in a Chinese Han population, which may have some implications for understanding the role of TLR4 in the pathophysiology of AAD.
patients [7–9]. More importantly, the local inflammation in the aortic wall and subsequent systemic inflammatory reaction were observed during the whole course of AAD [2, 10, 11]. As a consequence, investigating the inflammation-related pathogenic genes would be beneficial to understand the underlying mechanisms of AAD and to prevent and treat the disease.

Toll-like receptor 4 (TLR4), located on chromosome 9q32-q33, is not only the key pattern-recognition receptor in immune-inflammatory reactions but also the initiation protein in this signal transduction pathway [12]. Recently, the role of TLR4-mediated signaling has emerged in maintaining aortic homeostasis and establishing the aorta alterations (vascular remodeling and medial degeneration) and their complications [13, 14]. It was confirmed that the upregulation of TLR4 could evoke inflammatory cell infiltration, production of proinflammatory mediators, endothelial dysfunction, smooth muscle cell apoptosis, and aortic media degradation, which were closely related to aortic inflammation, remodeling, and dissection [15–17]. In our previous study, we preliminarily found that an elevated level of serum TLR4 expression was independently associated with the risk of AAD, and there was a positive relationship between serum TLR4 and circulating CRP [18]. The activity and function of TLR4 seem to be modulated by genetic variations, principally single nucleotide polymorphisms (SNPs), which may change TLR4 activity and thus modulate inflammatory reactions, thereby regulating the progression of various diseases [19, 20]. Therefore, it is reasonable to hypothesize that SNPs in the functional regions of the TLR4 gene may have effects on TLR4 activity and thus modify the signaling of immune and subsequent inflammatory responses, which in turn may affect AAD risk. Some evidences have exhibited that TLR4 polymorphisms were closely associated with the risk of infection [21], atherosclerotic disease [22], autoimmune disease [23], and tumor [24]. One study revealed that polymorphisms linked to the TLR4-mediated metalloproteinase pathways could obviously impact the risk of sporadic thoracic aortic aneurysm [25]. It remains unclear, however, whether TLR4 polymorphisms are relevant to the susceptibility of AAD.

In this case-control study, we aimed to discuss the correlation between TLR4 polymorphisms and AAD risk and examine whether potential gene-environment interactions could enhance the susceptibility to AAD. In addition, the consequences of these SNPs on the levels of AAD-related parameters and serum TLR4 in AAD patients were further investigated. Our study might contribute to the prediction of genetic variants associated with disease risk and add knowledge for the prevention and treatment for AAD.

### 2. Material and Methods

#### 2.1. Study Population

A total of 222 AAD patients and 222 controls were enrolled from the First Hospital of China Medical University from May 2017 to August 2018. Case and control participants were all Chinese Han population and matched by age and sex. All the patients were evaluated within 24 hours after symptom and diagnosed by the computed tomography angiography (CTA), and there were 172 patients under surgical treatment. Excluding criteria incorporated the subjects with coronary heart diseases, congenital cardiovascular defects, severe vascular stenosis, autoimmune diseases, severe organ failure, infectious diseases, hematological system diseases, or malignant tumors. Patients with certain genetic syndromes, such as Marfan syndrome and Ehlers-Danlos syndrome, or traumatic aneurysms were also excluded from the study. A 5 mL fasting venous blood sample was obtained from each subject for DNA isolation. The study was approved by the Ethics Committee of the First Hospital of China Medical University (Shenyang, China). Written informed consent was obtained from each participant.

#### 2.2. Data Collection

The demographic data and clinical related information were collected from participants’ medical records. And maximal aortic diameters of AAD subjects were assessed by CTA. Smoking was defined as having smoked at least one cigarette per day for more than one year. Drinking was defined as having consumed at least one alcoholic drink a day for a minimum period of six months. Body mass index (BMI) was computed as weight in kilograms divided by the square of height in meters. Obesity was defined as BMI ≥ 28 kg/m². Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg and/or use of antihypertensive medications. Diabetes was defined as fasting plasma glucose (FPG) ≥ 7 mmol/L (126 mg/dL) and/or under antidiabetic treatment. Dyslipidemia was defined as total cholesterol (TC) ≥ 6.22 mmol/L (240 mg/dL), or triglyceride (TG) ≥ 2.26 mmol/L (200 mg/dL), or high-density lipoprotein cholesterol (HDL-C) < 1.03 mmol/L (40 mg/dL), or low-density lipoprotein cholesterol (LDL-C) ≥ 4.14 mmol/L (160 mg/dL) and/or under taking hypolipidemic drugs.

#### 2.3. SNP Selection and Genotyping Assay

A two-step approach was adopted to identify tag-SNPs in TLR4 [26]. Briefly, we applied the combination of HapMap database (http://www.HapMap.org) and Haploview software 4.2 (http://www.broadinstitute.org/mpg/haplovew) to select the tag-SNPs, which should fit the following criteria: minor allele frequency (MAF) > 0.05 in Chinese Han population, low linkage disequilibrium ($r^2 < 0.8$), and Hardy-Weinberg equilibrium (HWE) > 0.05. Then, potential functions of tag-SNPs were predicted with SNPinfo Web Server (https://snpinfo.niehs.nih.gov/). Accordingly, rs10759932 in the promoter region and rs11536889 in the 3′-untranslated region (3′-UTR) of TLR4, which could separately modify the function of transcription factor binding sites and miRNA binding sites, were chosen in this study.

A routine phenol-chloroform method was utilized to extract genomic DNA from each blood clot. All samples were randomly placed on the 384-well plates and blinded for disease status. SNPs were genotyped by Baygene Biotechnology Company Limited (Shanghai, China) with the KASP method using the SNPLine platform (LGC, United Kingdom). Genotyping quality was assessed by repeated detection of 10% randomly selected samples, yielding a 100% concordance.
2.4. SNP-Gene Expression Correlation Analysis. Based on our previously published data [18], a total of 64 AAD patients with the information of serum TLR4 levels were involved in further genotype and TLR4 gene expression correlation analysis.

2.5. Statistical Analysis. All the data analyses were conducted with SPSS 17.0 software (SPSS Inc., Chicago, IL, United States). HWE for studied SNPs in each group was evaluated with the chi-square ($\chi^2$) test. Differences of baseline characteristics between AAD patients and controls were compared by the independent-sample $t$-test or $\chi^2$ test as appropriate. Comparisons of continuous variables among different genotype groups were performed with one-way ANOVA. The association of SNPs with AAD risk was estimated by calculating odds ratios (ORs) and their 95% confidence intervals (CIs) using multivariate logistic regression after adjusting the potential confounding factors. The log-likelihood ratio test was performed to evaluate the SNP-environment interaction by comparing the model that only involved the main effects with the full model also containing the interaction term. The Bonferroni correction was used to adjust $P$ values for multiple tests as needed. Moreover, the false-positive report probability (FPRP) was calculated to verify the significance of each association. Then, the FPRP values for multiple tests as needed. Moreover, the false-positive report probability (FPRP) was calculated to verify the significance of each association. Then, the FPRP values were figured out by following the published instructions, and only the significant result with FPRP < 0.5 was regarded as a noteworthy finding [27]. A two-sided $P < 0.05$ was considered statistically significant. In addition, the dominant and recessive genetic models were defined as heterozygote vs. homozygote variant and heterozygote vs. homozygote variant, respectively.

3. Results

3.1. Characteristics of the Study Population. Table 1 presents the baseline characteristics of the study participants. Compared with controls, AAD cases were not statistically different in age, sex, obesity, smoking, drinking, and dyslipidemia.

3.2. Association of TLR4 Polymorphisms with AAD Risk. The genotype distributions of rs10759932 and rs11536889 in each group are summarized in Table 2. The genotypes in controls were all in consistent with HWE ($P > 0.05$). After adjusting age, sex, obesity, smoking, drinking, hypertension, diabetes, and dyslipidemia, the rs10759932 CC genotype and recessive model were associated with a decreased risk of AAD with corresponding ORs of 0.393 (95%CI = 0.164-0.939, $P = 0.036$) and 0.439 (95%CI = 0.196-0.984, $P = 0.045$), respectively. The overall genetic effects for rs11536889 related to AAD were not observed.

To explore the correlation between TLR4 polymorphisms and AAD risk in specific subgroups, we further conducted stratified analyses on the basis of age and sex, as shown in Table 3. For rs10759932, the recessive model was associated with a reduced AAD risk in male subjects (OR = 0.343, 95% CI = 0.133-0.882, $P = 0.026$), and the heterozygote TC and dominant model conferred a decreased risk of AAD in female subjects (TC vs. TT: OR = 0.231, 95%CI = 0.071-0.752, $P = 0.015$; dominant model: OR = 0.241, 95%CI = 0.082-0.707, $P = 0.010$). As for rs11536889, its GC genotype and dominant model were significantly correlated with an increased risk of AAD in female subjects with OR values of 3.382 and 3.043 (all $P < 0.05$), respectively, compared with GG genotype.

3.3. The Interactions between TLR4 Polymorphisms and Risk Factors in AAD Susceptibility. The interaction effect between TLR4 polymorphisms and environmental factors on the risk of AAD was examined. A combined genotype including the dominant and recessive genetic models of TLR4 SNPs was used for interaction analysis. Table 4 showed that the most

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Table 1: Baseline characteristics of the study participants$^a$.

| Variables | Control ($n = 222$) | AAD ($n = 222$) | $P$ |
|-----------|-------------------|----------------|-----|
| Age (years) | 56.7 ± 11.8 | 56.3 ± 12.2 | 0.769 |
| Male, n (%) | 163 (73.4%) | 164 (73.9%) | 0.914 |
| Obesity | 0.081 |
| Yes, n (%) | 42 (18.9%) | 54 (24.3%) | |
| No, n (%) | 173 (77.9%) | 146 (65.8%) | |
| Missing, n (%) | 7 (3.2%) | 22 (9.9%) | |
| Smoking | 0.089 |
| Yes, n (%) | 81 (36.5%) | 73 (32.9%) | |
| No, n (%) | 112 (50.4%) | 143 (64.4%) | |
| Missing, n (%) | 29 (13.1%) | 6 (2.7%) | |
| Drinking | 0.065 |
| Yes, n (%) | 71 (31.9%) | 61 (27.5%) | |
| No, n (%) | 122 (55.0%) | 155 (69.8%) | |
| Missing, n (%) | 29 (13.1%) | 6 (2.7%) | |
| Hypertension | <0.001 |
| Yes, n (%) | 93 (41.9%) | 176 (79.3%) | |
| No, n (%) | 129 (58.1%) | 46 (20.7%) | |
| Diabetes | <0.001 |
| Yes, n (%) | 23 (10.4%) | 82 (36.9%) | |
| No, n (%) | 199 (89.6%) | 126 (56.8%) | |
| Missing, n (%) | — | 14 (6.3%) | |
| Dyslipidemia | 0.426 |
| Yes, n (%) | 100 (45.1%) | 105 (47.3%) | |
| No, n (%) | 121 (54.5%) | 109 (49.1%) | |
| Missing, n (%) | 1 (0.4%) | 8 (3.6%) | |
| WBC ($\times 10^9$/L) | — | 11.26 ± 4.46 | — |
| CRP (mg/L) | — | 77.00 ± 60.88 | — |
| D-dimer ($\mu g$/mL) | — | 4.65 ± 4.49 | — |
| Max. aortic diameter (cm) | — | 4.48 ± 0.95 | — |
| Serum TLR4 levels (ng/mL) | — | 13.31 ± 6.74 | — |

$^a$Demographic and clinical data for 222 AAD patients and 222 controls were collected from the medical records, and maximal aortic diameters of all cases were assessed by CTA. And serum TLR4 levels in a total of 64 AAD subjects were obtained from our previously published data [18].
significant interaction was between the rs11536889 recessive model and dyslipidemia and associated with an increased risk of AAD ($P_{\text{interaction}} = 0.038$, OR = 15.229), after the adjustment for age, sex, obesity, smoking, drinking, hypertension, diabetes, and dyslipidemia. NCBI Ref: reference frequencies of these SNPs in the Asian population (NCBI database). $P_{\text{corr}}$: $P$ values after Bonferroni correction. The results are in bold if $P < 0.05$.

3.4. FPRP Results. Now that AAD is a relatively rare disease and there are limited studies concerning the association between gene polymorphism and AAD risk, we set 0.5 as the prior probability of 0.25 or 0.1 (Table 5). It was shown that all of the significant findings for TLR4 rs11536889 polymorphism remained noteworthy at the prior probability of 0.25 or 0.1 (Table 5).

3.5. The Association of TLR4 Polymorphisms with Clinical Parameters and Serum TLR4 Levels in AAD Patients. Larger aortic diameters were observed in rs11536889 CC genotype carriers when compared to GG genotype carriers ($P = 0.037$) (Table 6). In addition, AAD subjects with CC genotype had significantly lower serum TLR4 levels than those with TT genotype for TLR4 rs10759932 ($P = 0.043$) (Table 6 and Figure 1).

4. Discussion

To our knowledge, no investigation has focused on the association between TLR4 polymorphisms and AAD susceptibility. The current study is the first report to identify the significance of TLR4 rs10759932 and rs11536889 polymorphisms and their interactions with environmental factors in the risk of AAD, as well as their associations with AAD-related clinical parameters and serum TLR4 levels.

AAD is one of the severe and major health issues of aortic disease known to be caused by inflammation, which could destroy the aortic structure and eventually lead to the aortic wall dissection and rupture [28]. TLR4 is considered a useful marker for evaluating local inflammatory reaction and has attracted particular interest because of its important function in mediating vascular remodeling and injury [15, 17]. As the most common form of genetic variation, SNPs in TLR4 functional regions might cause a dysfunction of TLR4 molecule and interfere with the host immunity and inflammation response, contributing to the risk of various diseases. The SNP rs10759932 locates in the promoter region of the TLR4 gene and may regulate the TLR4 expression level by influencing the binding affinity of transcription factors [29]. The T to C allele substitution of rs10759932 has been reported to be strongly associated with a reduced risk of malignant tumors [29, 30]. Similarly, our results indicated a significant association of rs10759932 CC genotype and recessive model with a reduced risk of AAD in the overall population, and the favorable effect of rs10759932 polymorphism on AAD was also prominent in the subgroup analyses stratified by sex. The rs11536889 polymorphism is located in the centre of the 2818-bp TLR4 3′-UTR, where a genetic change can influence mRNA stability and translation efficiency [24, 31]. A laboratory study by Sato et al. revealed that a fragment of 3′-UTR containing the TLR4 rs11536889 G allele, but not the C allele, inhibited luciferase activity triggered by LPS or IL-6 possibly by binding to miRNAs in posttranscriptional regulation [32]. Several studies found that TLR4 rs11536889 CC genotype or C allele was correlated with an increased risk of coronary artery disease [12], gastric cancer [33], and hepatitis A virus infection [34]. In this research, we figured out that female individuals carrying rs11536889 GC genotype or dominant model were more susceptible to AAD compared with those with GG genotype. The above findings implied that TLR4 rs10759932 and rs11536889 could be genetic biomarkers and potential therapeutic targets for AAD.

AAD is a complex trait, and its susceptibility may be enhanced by a combined effect of genetic background and

### Table 2: The association of TLR4 polymorphisms with the risk of AAD.

| SNP        | NCBI Ref | Control | AAD        | $P_{\text{corr}}$ | OR (95% CI) |
|------------|----------|---------|------------|-------------------|-------------|
| rs10759932 | $n = 28$ | $n = 222$ | $n = 222$ |                   |             |
| TT         | 13 (46.4%) | 93 (41.9%) | 106 (48.4%) | 0.297             | 0.759 (0.453-1.274) |
| TC         | 12 (42.9%) | 95 (42.8%) | 94 (42.9%) | 0.036 (0.072)     | 0.393 (0.164-0.939) |
| CC         | 3 (10.7%)  | 34 (15.3%) | 19 (8.7%)  | 0.045 (0.090)     | 0.439 (0.196-0.984) |
| CC+TC vs. TT |         |          |            | 0.112             | 0.673 (0.412-1.098) |
| CC vs. TC+TT |        |         |            |                   |             |
| $P_{\text{HWE}}$ | 0.239 | 0.775         |             |                   |             |

$P$ for association was adjusted by age, sex, obesity, smoking, drinking, hypertension, diabetes, and dyslipidemia. NCBI Ref: reference frequencies of these SNPs in the Asian population (NCBI database). $P_{\text{corr}}$: $P$ values after Bonferroni correction. The results are in bold if $P < 0.05$. 

### Table 6: Clinical Parameters and Serum TLR4 Levels in AAD Patients.

| Parameter | Control | AAD | $P_{\text{corr}}$ |
|-----------|---------|-----|-----------------|
| $n$       | $n = 28$ | $n = 222$ |             |
| Serum TLR4 | 5 (6.0%) | 5 (6.0%) | 0.598 |
| CC        | 3 (36.9%) | 57 (26.0%) | 0.934 |
| GC        | 48 (57.1%) | 154 (70.3%) | 0.775 |
| GG        | 13 (46.4%) | 93 (41.9%) | 1.351 |
| CC+GC vs. GG | 0.112 | 0.673 (0.412-1.098) | |
| CC vs. GC+GG | 0.045 | 0.439 (0.196-0.984) |
| $P_{\text{HWE}}$ | 0.351 | 0.004 |

$P_{\text{HWE}}$ for association was adjusted by age, sex, obesity, smoking, drinking, hypertension, diabetes, and dyslipidemia. NCBI Ref: reference frequencies of these SNPs in the Asian population (NCBI database). $P_{\text{corr}}$: $P$ values after Bonferroni correction. The results are in bold if $P < 0.05$. 

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TLR4: Toll-like receptor 4; AAD: Aortic dissection and rupture; NCBI: National Center for Biotechnology Information; HWE: Hardy-Weinberg equilibrium; OR: Odds Ratio; CI: Confidence Interval; P: Probability; corr: Corrected; FPRP: Functional Polymorphism Regional Prediction.
Table 3: Association of TLR4 polymorphisms with the risk of AAD stratified by age and sex.

| Genotypes | Control   | AAD       | \(P(P_{corr})\) | OR (95% CI) |
|-----------|-----------|-----------|------------------|-------------|
| rs10759932 |           |           |                  |             |
| Age       |           |           |                  |             |
| TT        | 50 (42.4%) | 61 (52.1%)| 0.464            | 0.786 (0.413-1.497) |
| TC        | 46 (39.0%) | 45 (38.5%)| 0.075            | 0.418 (0.160-1.093) |
| CC        | 22 (18.6%) | 11 (9.4%) | 0.075            | 0.472 (0.195-1.144) |
| >55y      |           |           |                  |             |
| TT        | 43 (41.3%) | 45 (44.1%)| 0.598            | 0.787 (0.323-1.917) |
| CC        | 12 (11.5%) | 8 (7.8%)  | 0.240            | 0.275 (0.032-2.371) |
| ≤55y      |           |           |                  |             |
| TT        | 43 (41.3%) | 45 (44.1%)| 0.015 (0.030)    | 0.241 (0.082-0.707) |
| CC        | 12 (11.5%) | 8 (7.8%)  | 0.253            | 0.365 (0.065-2.054) |
| Sex       |           |           |                  |             |
| Male      |           |           |                  |             |
| TT        | 66 (40.5%) | 67 (41.4%)| 0.560            | 1.198 (0.652-2.198) |
| TC        | 70 (42.9%) | 83 (51.2%)| 0.061            | 0.373 (0.133-1.049) |
| CC        | 27 (16.6%) | 12 (7.4%) | 0.935            | 0.976 (0.549-1.737) |
| Female    |           |           |                  |             |
| TT        | 27 (45.7%) | 39 (68.4%)| 0.015 (0.030)    | 0.231 (0.071-0.752) |
| CC        | 7 (11.9%)  | 7 (12.3%) | 0.590            | 1.729 (0.183-9.437) |
| rs11536889 |           |           |                  |             |
| Age       |           |           |                  |             |
| GG        | 83 (70.9%) | 78 (66.7%)| 0.935            | 1.029 (0.518-2.044) |
| GC        | 30 (25.6%) | 29 (24.8%)| 0.436            | 1.807 (0.408-8.009) |
| >55y      |           |           |                  |             |
| GG        | 71 (69.6%) | 70 (66.7%)| 0.907            | 1.064 (0.379-2.983) |
| GC        | 27 (26.5%) | 29 (27.6%)| 0.962            | 1.055 (0.116-9.595) |
| ≤55y      |           |           |                  |             |
| GG        | 71 (69.6%) | 70 (66.7%)| 0.941            | 0.965 (0.367-2.532) |
| GC        | 27 (26.5%) | 29 (27.6%)| 0.848            | 1.226 (0.153-9.824) |
| Sex       |           |           |                  |             |
| GG        | 114 (71.3%)| 115 (70.1%)| 0.384            | 0.746 (0.386-1.442) |
| GC        | 41 (25.6%) | 40 (24.4%)| 0.585            | 1.469 (0.369-5.839) |
| ≤55y      |           |           |                  |             |
| GG        | 40 (67.8%) | 33 (56.9%)| 0.041 (0.082)    | 3.382 (1.051-10.885) |
| GC        | 16 (27.1%) | 18 (31.0%)| 0.590            | 1.729 (0.236-12.673) |
| Female    |           |           |                  |             |
| GG        | 3 (5.1%)  | 7 (12.1%) | 0.042 (0.084)    | 3.043 (1.041-8.900) |
| GC        | 3 (5.1%)  | 7 (12.1%) | 0.786            | 1.313 (0.183-9.437) |

\(P\) for association was adjusted by obesity, smoking, drinking, hypertension, diabetes, and dyslipidemia. \(P_{corr}\): \(P\) values after Bonferroni correction. The results are in bold if \(P < 0.05\).
Table 4: The interaction effects between TLR4 polymorphisms and risk factors in the susceptibility to AAD.

| SNP genotypes | Number of subjects | Obesity | Smoking | Drinking | Hypertension | Dyslipidemia |
|---------------|--------------------|---------|---------|----------|--------------|--------------|
|               | No. of controls/cases | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes |
| rs10759932    | CC+TC              | 99/75  | 28/27  | 59/73  | 49/36  | 69/83  | 39/26  | 72/17  | 57/96  | 69/50  | 60/61  |
|               | OR (95% CI)         | 1.0 (ref.) | 1.234 (0.651-2.337) | 1.0 (ref.) | 0.564 (0.319-0.997) | 1.0 (ref.) | 0.497 (0.268-0.922) | 1.0 (ref.) | 7.250 (3.662-14.353) | 1.0 (ref.) | 1.433 (0.825-2.488) |
|               |                     | 74/69  | 14/26  | 53/69  | 32/35  | 53/71  | 32/33  | 57/29  | 36/77  | 52/57  | 40/43  |
|               | OR (95% CI)         | 0.954 (0.563-1.616) | 0.837 (0.456-1.537) | 1.065 (0.643-1.764) | 0.716 (0.388-1.322) | 1.657 (0.745-3.687) | 7.812 (3.829-15.940) | (0.769-2.405) | (0.478-2.533) |
| rs11536889    | TT                 | 40/12  | 10/4   | 16/11  | 12/8   | 19/13  | 9/6    | 20/2   | 14/17  | 20/8   | 14/11  |
|               | OR (95% CI)         | 0.768 (0.191-3.089) | 1.0 (ref.) | 0.889 (0.248-3.183) | 1.0 (ref.) | 0.768 (0.191-3.089) | 1.0 (ref.) | 10.045 (1.890-53.403) | 1.0 (ref.) | 3.667 (0.934-14.392) |
|               |                     | 149/132 | 32/49  | 96/131 | 69/63  | 103/141 | 62/53  | 109/44 | 79/156 | 101/99 | 86/93  |
| rs11536889    | TC+TT              | 53/50  | 10/15  | 33/51  | 28/19  | 38/51  | 23/19  | 41/20  | 24/54  | 33/45  | 32/27  |
|               | OR (95% CI)         | 1.620 (0.741-3.545) | 2.740 (1.141-6.579) | 2.197 (0.927-5.209) | 1.469 (0.604-3.572) | 2.213 (1.005-4.872) | 1.226 (0.531-2.831) | 16.174 (3.635-71.956) | 4.047 (1.300-12.603) | 13.888 |
|               | P_{interaction} = 0.495, OR = 1.838 (0.320-10.550) | P_{interaction} = 0.699, OR = 0.729 (0.147-3.616) | P_{interaction} = 0.353, OR = 0.433 (0.074-2.533) | |
| rs11536889    | GG                 | 117/96 | 32/39  | 79/92  | 51/54  | 84/104 | 46/42  | 86/26  | 68/122 | 87/64  | 66/78  |
|               | OR (95% CI)         | 1.473 (0.833-2.605) | 1.0 (ref.) | 0.886 (0.532-1.473) | 1.0 (ref.) | 0.623 (0.363-1.068) | 1.0 (ref.) | 7.041 (3.785-13.097) | 1.0 (ref.) | 1.545 (0.938-2.548) |
|               |                     | 53/50  | 10/15  | 33/51  | 28/19  | 38/51  | 23/19  | 41/20  | 24/54  | 33/45  | 32/27  |
| rs11536889    | CC+GC              | 1.049 (0.638-1.718) | 1.561 (0.659-3.694) | 1.333 (0.760-2.337) | 0.583 (0.295-1.154) | 1.061 (0.622-1.809) | 0.604 (0.298-1.223) | 1.618 (0.724-3.616) | 7.596 (3.651-15.802) | 1.500 (0.824-2.729) | 1.972 |
|               | OR (95% CI)         | 0.537 (0.357-0.714) | 0.714 (0.425-1.207) | 0.245-2.078 |

P_{interaction} = 0.537, OR = 0.714 (0.245-2.078)
Table 4: Continued.

| GC+GG | P<sub>interaction</sub> = 0.870, OR = 0.114 (0.305-4.069) | P<sub>interaction</sub> = 0.052, OR = 0.346 (0.118-1.011) | P<sub>interaction</sub> = 0.273, OR = 0.531 (0.172-1.646) | P<sub>interaction</sub> = 0.095, OR = 0.413 (0.146-1.166) |
|-------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| No. of controls/cases | 87/164 | 113/101 | 164/136 | 41/51 | 106/134 | 78/67 | 116/143 | 68/58 | 124/42 |
| OR (95% CI) | 1.463 (0.895-2.393) | 1.064 (0.431-2.022) | 1.0 (ref.) | 1.063 (0.384-0.948) | 1.0 (ref.) | 5.873 | 1.071 | (0.714-1.605) |
| CC | P<sub>interaction</sub> = 0.999, OR = 0.999 (0.066-15.184) | P<sub>interaction</sub> = 0.170, OR = 6.289 (0.456-86.754) | P<sub>interaction</sub> = 0.515, OR = 2.700 (0.136-53.671) | P<sub>interaction</sub> = 0.865, OR = 1.276 (0.076-21.291) |
| No. of controls/cases | 6/10 | 1/3 | 6/9 | 1/6 | 6/12 | 1/3 | 3/4 | 5/12 | 7/8 | 1/8 |
| OR (95% CI) | 1.805 (0.575-5.660) | 1.053 (0.312-3.554) | 4.386 (0.504-38.175) | 1.571 (0.511-4.829) | 1.746 | 1.613 | 8.065 (2.363-27.523) | 1.143 | 7.838 |
| P for association was adjusted by age, sex, smoking, drinking, hypertension, diabetes, and dyslipidemia. bP for association was adjusted by age, sex, obesity, drinking, hypertension, diabetes, and dyslipidemia. cP for association was adjusted by age, sex, obesity, smoking, drinking, hypertension, diabetes, and dyslipidemia. dP for association was adjusted by age, sex, obesity, smoking, drinking, hypertension, and diabetes. eP for association was adjusted by age, sex, obesity, smoking, drinking, hypertension, and diabetes. #P value after Bonferroni correction. The results are in bold if P < 0.05.
Table 5: FRPR values for significant results on associations between TLR4 polymorphisms and AAD risk.

| Genotype                          | Number of controls/cases | OR (95% CI)       | P       | Statistical power | 0.25 | 0.1  | 0.01  | 0.001  | 0.0001 |
|-----------------------------------|--------------------------|-------------------|---------|-------------------|------|------|-------|-------|--------|
| rs10759932                        |                          |                   |         |                   |      |      |       |       |        |
| CC vs. TT (overall)               | 34/19 vs. 93/106         | 0.393 (0.164-0.939) | 0.036   | <0.001            | 0.995 | 0.998 | 0.999 | 1.000 | 1.000  |
| CC vs. TC+TT (overall)            | 34/19 vs. 188/200        | 0.439 (0.196-0.984) | 0.045   | <0.001            | 0.996 | 0.998 | 0.999 | 1.000 | 1.000  |
| CC vs. TC+TT (male)               | 27/12 vs. 136/150        | 0.343 (0.133-0.882) | 0.026   | <0.001            | 0.993 | 0.996 | 0.999 | 1.000 | 1.000  |
| TC vs. TT (female)                | 25/11 vs. 27/39          | 0.231 (0.071-0.752) | 0.015   | <0.001            | 0.987 | 0.994 | 0.999 | 1.000 | 1.000  |
| CC+TC vs. TT (female)             | 32/18 vs. 27/39          | 0.241 (0.082-0.707) | 0.010   | <0.001            | 0.981 | 0.991 | 0.999 | 1.000 | 1.000  |
| rs11536889                        |                          |                   |         |                   |      |      |       |       |        |
| GC vs. GG (female)                | 16/18 vs. 40/33          | 3.382 (1.051-10.885) | 0.041   | 0.706              | 0.236 | 0.392 | 0.854 | 0.983 | 0.998  |
| CC+GC vs. GG (female)             | 19/25 vs. 40/33          | 3.043 (1.041-8.900) | 0.042   | 0.705              | 0.241 | 0.398 | 0.857 | 0.984 | 0.998  |
| CC vs. GC+GG (interaction with dyslipidemia) | 1/8 vs. 113/101 | 15.229 (1.156-200.621) | 0.038 | 0.897 | 0.184 | 0.320 | 0.811 | 0.977 | 0.998 |

*The statistical power was calculated using the number of observations, ORs, and P values in this table. The results are in bold if FPRP < 0.5.
Table 6: Association of TLR4 polymorphisms with clinical parameters and serum TLR4 levels in AAD patients.

| Variables                  | TT (n = 106) | rs10759932 (n = 94) | CC (n = 19) | GG (n = 148) | rs11536889 (n = 58) | CC (n = 16) |
|----------------------------|--------------|---------------------|-------------|--------------|---------------------|-------------|
| WBC (×10³/L)               | 10.84 ± 4.35 | 11.63 ± 4.39        | 11.48 ± 5.43 | 11.06 ± 4.47 | 11.80 ± 3.94        | 11.07 ± 6.12 |
| CRP (mg/L)                 | 77.03 ± 65.71| 76.57 ± 54.78       | 71.58 ± 63.35 | 79.51 ± 63.79 | 72.95 ± 55.34       | 69.51 ± 55.72 |
| D-dimer (μg/mL)            | 4.39 ± 4.24  | 4.68 ± 4.49         | 6.34 ± 6.17  | 4.76 ± 4.57  | 4.81 ± 4.66         | 2.95 ± 2.40  |
| Max. aortic diameter (cm)  | 4.40 ± 0.86  | 4.55 ± 1.06         | 4.69 ± 0.76  | 4.40 ± 0.82  | 4.55 ± 1.12         | 4.96 ± 1.12  |
| Serum TLR4 levels (ng/mL)  | 14.83 ± 7.25 | 12.35 ± 5.56        | 9.78 ± 6.13* | 13.97 ± 7.69 | 13.07 ± 5.69        | 10.83 ± 3.56 |

*P < 0.05 vs. wild-type.

Figure 1: The effect of TLR4 rs10759932 and rs11536889 polymorphisms on serum TLR4 levels. *P < 0.05.

In summary, our data demonstrated that TLR4 rs10759932 was a protective factor whereas rs11536889 was a risk factor for AAD in a Chinese Han population, and these genetic correlations were independent of the classical cardiovascular risk factors. The interaction between rs11536889 recessive model and dyslipidemia enhanced the susceptibility to AAD. Furthermore, rs11536889 had a significant impact on AAD size, and rs10759932 was in an evident association with serum TLR4 levels.

5. Conclusion

In summary, our data demonstrated that TLR4 rs10759932 was a protective factor whereas rs11536889 was a risk factor for AAD in a Chinese Han population, and these genetic correlations were independent of the classical cardiovascular risk factors. The interaction between rs11536889 recessive model and dyslipidemia could enhance the susceptibility to AAD. Furthermore, rs11536889 had a significant impact on AAD size, and rs10759932 was in an evident association with serum TLR4 expression levels. Our findings may provide context for the better understanding of genetic features of AAD and thus facilitate the improvement of diagnostic and therapeutic approaches for AAD patients.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors state that they have no competing interests.
Authors’ Contributions

Tan Li performed the experiments, conducted statistical analysis, and wrote the manuscript. Xiaozheng Liu and Hongxia Ning collected the blood samples. Xintong Li contributed to the collection of clinical data. Jun Yang participated in the research design. Chunyan Ma designed the research and revised the manuscript. All authors read and approved the final manuscript.

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