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*Original Article*

# *SERPINE1* polymorphisms and the susceptibility of avascular necrosis of femur head

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**Abstract:** Purpose: This study was aimed to detect the association of rs11178 and rs2227631 polymporphisms in serpin peptidase inhibitor, clade E (*SERPINE1*) gene with avascular necrosis of femur head (ANFH) susceptibility. Methods: 106 ANFH patients and 109 healthy controls were enrolled in this case-control study. *SERPINE1* gene rs11178 and rs2227631 polymporphisms were genotyped by MALDI-TOF mass spectrometer. Linkage disequilibrium and haplotypes of the two polymorphisms were detected by Hploview. $X^2$ test and logistic regression analysis were used to calculate the frequencies differences of genotypes, alleles and haplotypes between case and control groups. Association strength of these polymorphisms with ANFH risk was represented by odds ratios (ORs) with 95% confidence intervals (CIs). Results: Genotypes and alleles of rs11178 in *SERPINE1* gene had no apparent association with the occurrence of ANFH ($P$>0.05). AA genotype and A allele of rs2227631 significantly increased the risk of ANFH ($P$=0.024, OR=2.363, 95% CI=1.110-5.028; $P$=0.016, OR=1.606, 95% CI=1.090-2.367). Moreover, after adjusting age, gender and BMI, AA genotype of rs2227631 also showed significant association with risk of ANFH (OR=2.352, 95% CI=1.091-5.068). Linkage disequilibrium analysis showed that rs11178 and rs2227631 constituted four haplotypes. Among these haplotypes, only T-A was significantly associated with ANFH risk ($P$=0.016, OR=2.271, 95% CI=1.154-4.469). Conclusion: *SERPINE1* gene rs2227631 polymorphism apparently associated with ANFH occurrence. Haplotypes of rs11178 and rs2227631 also related to ANFH.

**Keywords:** ANFH, *SERPINE1*, polymorphisms, haplotypes

## Introduction

Avascular necrosis of femur head (ANFH), also known as osteonecrosis of the femoral head (ONFH), is one of the frequently observed diseases in orthopedic clinic. Owing to abnormal blood supply, disorder of fibrinolytic system and other factors, there appear hip joint chondronecrosis and collapse and degeneration of femoral head. Recently, ANFH morbidity is rising year by year and the onset age trends to be younger [1]. This disease has difficult early diagnosis and poor treatment effect. The mild symptoms of many ANFH patients can be abated after therapy. For the serious patients, the operation of artificial joint replacement is finally adopted, which causes great suffering and economic burden [2-4]. Exploration of ANFH pathogenesis will provide theoretical basis for the treatment of this disease. Recent years, several studies have shown that many factors take part in the occurrence and development of ANFH, including excessive drinking, abuse of hormone, trauma, genetic and other factors [5, 6]. Among these factors, genetic factors play an important role in the occurrence of ANFH, especially to individuals differences. However, the pathogenesis of ANFH has not been figured out clearly.

Recently, there is a widespread agreement that fibrinolytic system disorder is the main pathogenesis of ANFH. Plasminogen activator inhibitor-1 (PAI-1), a regulatory factor of fibrinolytic system, shows significant association with ANFH [7]. PAI-1, also called serine protease inhibitor E (SERPINE1), is a kind of glycoprotein composed by 379 amino acids. In human, PAI-1 is encoded by *SERPINE1* gene which is located on the seventh chromosome 7q21.1. PAI-1 can decrease protein aggregation which is caused by fibrin degradation, maintaining

**Table 1.** Primer information of *SERPINE1* polymorphisms

| SNP | Primer type | Primer sequence |
|---|---|---|
| rs11178 (3'UTR) | PCR | Forward: 5'-ACGTTGGATGATAGTTTCTACCAGGCACAC-3' |
| | | Reverse: 5'-ACGTTGGATGAGACCTGGTTCCCACTGAG-3' |
| | Extension | Forward: 5'-AGGCCCTTTGCAGGAC-3' |
| | | Reverse: 5'-AGGCCCTTTGCAGGAT-3' |
| rs2227631 (promoter) | PCR | Forward: 5'-ACGTTGGATGAAGGAAACAGGAGACCAACG-3' |
| | | Reverse: 5'-ACGTTGGATGAGGATAAAGGACAAGCTGCC-3' |
| | Extension | Forward: 5'-GCATAGCGGGCAGCTCGAA-3' |
| | | Reverse: 5'-GCATAGCGGGCAGCTCGAG-3' |

**Table 2.** General data of study subjects

| Clinical features | Cases (n=106) | Controls (n=109) | *P* |
|---|---|---|---|
| Age (year) | 41.56±6.27 | 40.25±5.42 | 0.100 |
| Gender (male/female) | 56/50 | 61/48 | 0.645 |
| BMI (kg/m$^2$) | 23.44±3.76 | 22.75±3.23 | 0.150 |

Note: BMI: body mass index.

the dynamic equilibrium of normal coagulation in blood and fibrinolytic system. It is reported that PAI-1 may be related to ANFH [8-10]. However, the role of *SERPINE1* gene acts in the pathogenesis of ANFH still unclear. Previous studies about the association between *SERPINE1* gene and ANFH mainly concentrate on single nucleotide polymorphisms (SNPs) [11, 12].

*SERPINE1* gene rs11178 and rs2227631 polymorphisms were chosen in this study, in order to clarify the pathogenesis of ANFH. The detection of their associations with ANFH susceptibility will supply a theoretical basis for the improvement of the clinical diagnosis level of ANFH.

## Materials and methods

### *Research objects*

A total of 106 ANFH patients and 109 healthy controls in Shandong Provincial Hospital Affiliated to Shandong University in Shandong Province from 2012 to 2015 were recruited in this case-control study. After conducted with X-ray and bone scanning, the patients were diagnosed with ANFH or without any bone trauma. In the controls, there were no hereditary diseases and abnormal blood fat, blood routine and electrocardiogram. The cases were matched with the controls in residence, nationality, age and gender. All the participants signed the informed consent form. This study was approved by the ethic committee of Shandong Provincial Hospital Affiliated to Shandong University.

### *DNA extraction*

Peripheral blood which was extracted from every participant was processed with EDTA. Genomic DNA was extracted by DNA isolation kit (Sigma, USA). Ultraviolet spectrophotometer was applied to test the purity and concentration of the DNA samples. The qualified samples were preserved in -20°C for spare.

### *SNP genotyping*

Primers for *SERPINE1* gene polymorphisms were synthesized by Shanghai Sangon Biotech Co., Ltd (**Table 1**). MALDI-TOF mass spectrometer (Bruker Daltonics, Germany) was adapted to conduct SNP genotyping according to the manufacturer's instructions.

### *Statistical analysis*

Calculations were performed by SPSS 18.0 software. Clinical features were assessed by *t* test. Hardy-Weinberg equilibrium (HWE) was applied to carry out the goodness of fit of subjects. Haploview was used to analyze the linkage disequilibrium and haplotypes between *SERPINE1* gene rs11178 and rs2227631 polymorphisms. Comparisons of genotype, allele and haplotype between the case and control groups were conducted by $X^2$ examination. Relative risk of ANFH was assessed by odds radios (ORs) with 95% confidence intervals (95% CIs). Logistic regression analysis was applied to evaluate the contribu-

**Table 3.** Distributions of genotypes and alleles of rs11178 and rs2227631

| Locus | Genotype/Allele | Cases n=106 | Controls n=109 | OR (95% CI) | *P* |
|---|---|---|---|---|---|
| rs11178 | TT | 33 (31.13) | 37 (33.95) | 1.00 | |
| | CT | 46 (43.40) | 51 (46.79) | 1.011 (0.546-1.872) | 0.971 |
| | CC | 27 (25.47) | 21 (19.27) | 1.442 (0.689-3.017) | 0.331 |
| | T | 112 (52.83) | 125 (57.34) | 1.00 | |
| | C | 100 (47.17) | 93 (42.66) | 1.200 (0.820-1.756) | 0.347 |
| | HWE | | *P*=0.649 | | |
| rs2227631 | GG | 35 (15.1) | 49 (44.95) | 1.00 | |
| | AG | 44 (38.7) | 44 (40.37) | 1.400 (0.767-2.557) | 0.273 |
| | AA | 27 (46.2) | 16 (14.68) | 2.363 (1.110-5.028) | 0.024 |
| | G | 114 (53.8) | 142 (65.14) | 1.00 | |
| | A | 98 (46.2) | 76 (34.86) | 1.606 (1.090-2.367) | 0.016 |
| | HWE | | *P*=0.246 | | |

**Table 4.** The contributions of genotypes of rs11178 and rs2227631 for ANFH risk after adjusting age, gender and BMI value

| Locus | Genotype | OR* (95% CI) | *P** |
|---|---|---|---|
| rs11178 | TT | 1.00 | - |
| | CT | 0.961 (0.514-1.796) | 0.900 |
| | CC | 1.504 (0.706-3.204) | 0.290 |
| rs2227631 | GG | 1.00 | - |
| | AG | 1.40 (0.760-2.581) | 0.280 |
| | AA | 2.352 (1.091-5.068) | 0.029 |

tions of genotypes of rs11178 and rs2227631 on ANFH risk after adjusting age, gender and BMI. *P*<0.05 means statistical significance.

## Results

*Clinical features and HWE test*

Clinical features of participants were shown in **Table 2**. As for age, gender and body mass index (BMI), there were no apparent differences in the two groups (*P*>0.05). Genotypes distributions of *SERPINE1* rs11178 and rs2227631 polymorphisms all corresponded with HWE in controls, which suggested that the objects were representative (**Table 3**).

The genotyping of *SERPINE1* rs11178 and rs2227631 SNPs was performed by MALDI-TOF mass spectrometer. Genotype and allele frequencies of rs11178 and rs2227631 SNPs in *SERPINE1* gene were shown in **Table 3**. No evident differences of the genotype and allele frequencies of rs11178 were observed between case and control groups (*P*>0.05). Although AG genotype frequency of rs2227631 was different in the two groups, but the difference was not obviously. AA genotype frequency was apparently higher in cases, indicating a significant association with the susceptibility of ANFH (*P*=0.024, OR=2.363, 95% CI=1.110-5.028). Furthermore, after adjusting age, gender and BMI value, AA genotype of rs2227631 also showed significant association with ANFH risk (OR=2.352, 95% CI=1.091-5.068, P=0.029) (**Table 4**). Mutational allele A of rs2227631 may significantly increased the risk of ANFH (*P*=0.016, OR=1.606, 95% CI=1.090-2.367).

*Haplotype analysis of rs11178 and rs2227631*

Linkage disequilibrium existed between rs11178 and rs2227631 polymorphism ($D'$=0.765, $r^2$=0.506). Four haplotypes were constituted by the two SNPs (**Table 5**). T-A and C-A haplotypes were more frequently discovered in cases than in controls. But compared with T-G haplotype, only T-A haplotype significantly increased the susceptibility of ANFH (*P*=0.016, OR=2.271, 95% CI=1.154-4.469).

## Discussion

ANFH is a kind of orthopedic disease of collapse in the femur head. This disease is caused by degeneration and necrosis of bone trabecula and cartilages which is due to supply break of blood [5, 13]. Studies have shown that ANFH is not only a femoral head lesion but also a kind of multisystem disease [14]. As a complex disease, ANFH should be affected by genetic

**Table 5.** Haplotype analysis of rs11178 and rs2227631

| Haplotype | Cases (2 n=212) | Controls (2 n=218) | OR (95% CI) | $\chi^2$ | P |
|---|---|---|---|---|---|
| T-G | 84 (39.62) | 109 (50.00) | 1.00 | - | - |
| T-A | 28 (13.21) | 16 (7.34) | 2.271 (1.154-4.469) | 5.815 | 0.016 |
| C-G | 30 (14.15) | 33 (15.14) | 1.180 (0.667-2.087) | 0.323 | 0.570 |
| C-A | 70 (33.02) | 60 (27.52) | 1.514 (0.968-2.367) | 3.318 | 0.069 |

and environmental factors [5]. However, ANFH pathogenesis has not been figured out clearly up to now. Family based and twins based studies have pointed out that individuals with the similar genetic information will get the analogous susceptibility to the same disease [15-18], suggesting genetic factors play a crucial role in disease occurrence.

In healthy individuals, a tightly controlled balance exists between thrombosis and fibrinolysis. As a crucial enzyme in fibrinolytic system, PAI-1 takes part in the combination of plasmin activators and fibrinolytic balance. Besides, disorder blood supply is the leading cause of ANFH. So it is surmised that PAI-1 contribute to ANFH occurrence. Association of PAI-mediated fibrinolysis with osteonecrosis is firstly described by Nilsson et al. [19]. Subsequently, a lot of researches explore this association, and then many investigators report that PAI-1 closely relate to osteonecrosis [20, 21].

Genetic factors which influence the development of disease mainly manifests in genetic polymorphisms. Polymorphisms take an important part in the occurrence, treatment and prognosis of diseases. The most common mutation in genes is SNP which is the third generation biomarker in the early diagnosis and treatment for diseases. PAI-1, an important enzyme in human healthy, is encoded by *SERPINE1* gene. Recently, *SERPINE1* polymorphisms have been found to be associated with more and more diseases [22-26]. Subsequently, Kim et al. [27] have found that three polymorphisms (rs2227631, rs1799889 and rs11178) in *SERPINE1* as well as the A-4G-C haplotype of them all apparently increase the risk of ANFH in Korea population. However, the role of *SERPINE1* gene polymorphisms in the occurrence of ANFH is still unclear. Moreover, for the different region, ethnicity and lifestyle, polymorphism distributions exist differences.

According to above evidence, we detected the associations of rs11178 and rs2227631 polymorphisms as well as their haplotypes with the risk of ANFH. No apparent association was observed between rs11178 and ANFH susceptibility in present study. AA genotype and A allele of rs2227631 were significantly related to the risk of ANFH, with the increased risk of approximately 2.363 and 1.606 times. In order to get an accurate result which with respect to *SERPINE1* gene polymorphisms (rs1-1178 and rs2227631) and ANFH risk, we carried out the linkage disequilibrium and haplotype analysis. Then the results showed that T-A haplotype of these SNPs may be risk factor for ANFH onset, which associated with 2.271 times increased risk of ANFH. The result of this study was partly agreed with previous studies [5, 26].

Although we obtained a positive result which provided a certain theory reference for the diagnosis and treatment of ANFH, it was not yet sufficient to certify the role of *SERPINE1* gene in the pathogenesis of this disease. Genotype and allele distributions of the two SNPs were not diverged from HWE, indicating the representativeness of the subjects. But some limitations in our study should not be neglect. Firstly, sample size was small. Secondly, other genetic factors and environmental factors may be influenced the incidence of ANFH. Present result was not adjusted by confounding factors. Thirdly, only one ethnicity recruited from one region implicated in this study. All of the above reasons might lead to an unfaithful result.

To sum up, the pathogenesis of ANFH should be further clarified and improved in later studies, which include a large sample size, confounding factors and more ethnicity groups.

## Disclosure of conflict of interest

None.

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