

Original Article

ABCB1 polymorphisms associated with osteonecrosis of the femoral head

Zongyu Zhang¹, Yawei Li¹, Huaiying Liu¹, Jinhui Shi², Xuefeng Li², Weimin Jiang²

¹Lianyungang Affiliated Hospital of Nanjing University of Chinese Medicine, China; ²1st Affiliated Hospital of Suzhou University, China

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Abstract: Aims: This case-control study was conducted to investigate the relation of ATP-binding cassette subfamily B member 1 (*ABCB1*) C1236T and C3435T polymorphisms and non-traumatic osteonecrosis of the femoral head (ONFH). Methods: We gathered 113 ONFH patients and 116 controls in the study. The polymorphisms of *ABCB1* were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technology. Odds ratio (OR) with 95% confidence interval (CI) were adopted to analyze the correlation between *ABCB1* polymorphisms and ONFH. Results: In the study, we found that the frequency of C3435T TT genotype was much lower in case group, compared with that of controls (17.7% vs. 23.3%). Moreover, OR and 95% CI values indicated that C3435T TT genotype served as a protective factor for ONFH (OR=0.34, 95% CI=0.15-0.75). Meanwhile, the risk for the T allele carriers was much lower than C allele (OR=0.60, 95% CI=0.42-0.87). However, C1236T polymorphism showed no significant effects on the pathogenesis of ONFH. In the haplotype analysis, T-T haplotype appeared to be an inhibitor for ONFH (OR=0.45, 95% CI=0.23-0.87). Conclusions: Based on the results, *ABCB1* polymorphisms were associated with the risk for ONFH.

Keywords: *ABCB1*, polymorphisms, ONFH

Introduction

Osteonecrosis of the femoral head (ONFH), also called ischemic ONFH, is a common disease caused by various factors, such as corticosteroid, alcohol, smoking, HIV infection and genetic mutations [1-8]. As we all know, ischemia of osseous tissue could result in ischemic necrosis in bones. ONFH at early stage was characterized by the damage in a single main blood vessel, and as the conditions worsen, circulating blood cannot satisfy the need of osteocytes, myeloid tissues were damaged and necrosis appears in osteocytes.

Both genetic and environmental factors play critical roles in the pathogenesis of ONFH, but it has been generally acknowledged that only under certain genetic background, can environmental factors affect the onset of diseases [9-11]. Moreover, existing data have verified that the occurrence of ONFH is closely correlated with aberrant expression of genes [12-18]. Among them, ATP-binding cassette sub-

family B member 1 (*ABCB1*) gene serves as a tumor suppressor.

P-glycoprotein (P-gp), encoded by *ABCB1* gene, plays crucial role in drugs treatment [19]. Additionally, there is relationship of P-gp and ONFH risk, the increased P-gp activity indicating low risk for ONFH [20]. For *ABCB1* gene, it has been demonstrated that several SNPs could regulate expression level of P-gp. A single SNP shows relatively weak effect on the disease, while the combination of multiple SNPs could make a person more likely to suffer certain disease [21-23]. In the study, we selected two SNPs (C1236T and C3435T) in *ABCB1* gene and analyzed the association of genotypes and haplotypes of C1236T and C3435T polymorphisms with ONFH susceptibility.

Materials and methods

Study population

A total of 113 non-traumatic ONFH patients (71 males, 42 females, and mean age 42.3±11.5)

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Table 1. Primer sequences

SNP	Primers	Primer sequence	Length of amplified fragments/bp	Restriction enzyme
C1236T	Forward	5'-TTTCTCACTCGTCTGGTA-3'	502	Eco0109I
	Reverse	5'-GTCATAGGCATTGGCTTC-3'		
C3435T	Forward	5'-TGTGCCTGGTCTGAAGTT-3'	246	Dpn
	Reverse	5'-TAGGCAGTGA CTGATGAA-3'		

Reagents

Genomic DNA Extraction Kit (Tiangen Biotech (Beijing) Co., Ltd, DP318-03); DNA Molecular Weight Marker (DNA100bp Marker); Restriction enzyme Eco0109I and Dpn II; Platinum Taq DNA polymerase (Shanghai Invitrogen Biotech Co., Ltd).

in Chinese Han population were collected from Lianyungang Affiliated Hospital of Nanjing University of Chinese Medicine. All the cases conformed to the diagnostic criteria of ONFH put forward by Mont et al. [24].

The diagnostic criteria of ONFH were as follows: (1) Clinical symptoms, signs and medical history: arthralgia accompanied with knee pains in the main sites including groin, hip and thigh; limited internal rotation of the hip joint; histories of hip injury, corticosteroid use, alcoholism and diving. (2) Banding low signal intensity on T1 weighted MRI or double-line sign on T2 weighted images. (3) Changes of the X-ray film: signs of water drop, low density, crescent, breakage, sclerosis, deformation and reparation. (4) CT results reveal unequal-sized sacate photic zones with obscure margins and high-density sclerotic bones in the femoral head; irregular deformations of fracture and collapse of the femoral head; narrowed or obliterated gap of the hip joint. (5) Blood flow perfusion at early stage of ONFH though bone scintigraphy indicates perfusion defects (non-radiation region); blood-pool phase of necrosis and regeneration shows bagel-like changes of the non-radiation region in the hot area. (6) DSA radiography manifests tiny or discontinuous feeding arteries of the superior retinaculum, or the reflux of femoral veins delays the sedimentation of contrast media. (7) According to the bone biopsy, the bone trabecula with empty lacunae accounting for over 50% of osteocytes affects some other bone trabeculae nearby and leads to bone marrow necrosis. Individuals meeting at least two of the above criteria can be diagnosed as ONFH. In contrast, 116 controls (66 males, 50 females, mean age 40.1±11.3) were all healthy people visiting the same hospital and were comparable with the cases in age and gender. The written informed consent was obtained from each subject and the study was approved by Ethical Committee.

Genomic DNA extraction

Peripheral venous blood (EDTA anticoagulation) was gathered from the subjects. Then we extracted DNA using phenol-chloroform method, measured DNA density with ultraviolet spectrophotometry, and stored DNA samples at -80°C.

Polymerase chain reaction-restricted fragment length polymorphisms (PCR-RFLP)

PCR-RFLP technology was adopted to distinguish the genotypes of *ABCB1* C1236T and C3435T polymorphisms. The primers sequences, restriction enzyme and digestion segments were shown in **Table 1**. PCR amplification was conducted with a 20 µL solution including 10×PCR reaction buffer 2 µL, dNTP 2 µL (2.5 mmol/L), forward and reverse primer respectively 0.5 µL (10 pmol/µL), Platinum Taq DNA polymerase 2.5 U and genomic DNA 50 ng. As for *ABCB1* C1236T, PCR cycle parameters were as follows: initial denaturation 94°C for 5 min, 35 cycles of denaturation 94°C for 30 s, annealing 54°C for 1 min, extension 72°C for 1 min, and finally 72°C extension for 7 min. The fragment of PCR products was 502 bp. As for *ABCB1* C3435T, the procedure was as follows: initial denaturation 94°C for 3 min, 35 cycles of denaturation 94°C for 40 s, annealing 52°C for 1 min, extension 72°C for 1 min, and 72°C extension for 7 min. The fragment of PCR products was 246 bp. Then PCR products were digested by Eco0109I and Dpn II, respectively. The digested products were detected by 2% agarose gel electrophoresis.

Statistical methods

All the statistical analyses were done with SPSS 18.0 software and the significance level was set at $P < 0.05$. χ^2 test was applied to check the

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Table 2. Genotypes and alleles distribution of *ABCB1* C1236T and C3435T polymorphisms

Genotype/allele	Case (n, %)	Control (n, %)	χ^2	P value	OR (95% CI)
C1236T					
CC	27 (23.9)	25 (21.6)	-	-	1.000
CT	53 (46.9)	60 (51.7)	0.359	0.549	0.82 (0.42-1.58)
TT	33 (29.2)	31 (26.7)	0.001	0.969	0.99 (0.47-2.05)
C	107 (47.3)	110 (47.4)	-	-	1.000
T	119 (52.7)	122 (52.6)	0.000	0.988	1.00 (0.70-1.45)
C3435T					
CC	43 (40.0)	28 (24.1)	-	-	1.000
CT	56 (51.3)	61 (52.6)	2.859	0.091	0.60 (0.33-1.09)
TT	14 (17.7)	27 (23.3)	7.258	0.007	0.34 (0.15-0.75)
C	142 (62.8)	117 (50.4)	-	-	1.000
T	84 (37.2)	115 (49.6)	7.165	0.007	0.60 (0.42-0.87)

Table 3. Haplotypes analysis for *ABCB1* C1236T and C3435T polymorphism

Haplotype 1-2	Case (2n=226, %)	Control (2n=232, %)	χ^2	P value	OR (95% CI)
C-C	47 (20.8)	35 (15.1)	-	-	1
C-T	60 (26.5)	75 (32.3)	3.382	0.066	0.60 (0.34-1.04)
T-C	95 (42.0)	82 (35.3)	0.301	0.584	0.86 (0.51-1.46)
T-T	24 (10.6)	40 (17.2)	5.651	0.017	0.45 (0.23-0.87)

compliance degree of genotypes distribution of each SNP with Hardy Weinberg Equilibrium (HWE). The differences of genotypes and haplotypes distribution between case and control group were analyzed by χ^2 test. Odds ratio (OR) and 95% confidence interval (CI) were used to evaluate the association of *ABCB1* polymorphisms with ONFH risk.

Results

Population characteristics

For the cases and controls, genotype distribution of *ABCB1* C1236T and C3435T accorded with HWE, indicating the selected subjects were well representative.

Analysis for correlation of *ABCB1* C1236T and C3435T polymorphisms with ONFH

Genotypes and alleles frequencies of *ABCB1* C1236T and C3435T were displayed in **Table 2**. The results showed that TT genotype of C3435T showed strongly protective effects on the occurrence of ONFH (OR=0.34, 95% CI=0.15-0.75). Moreover, we found that fre-

quency of C3435T allele was 37.2% in cases, significantly lower than that of control group (49.6%), which suggested that T allele could inhibit the onset of ONFH (OR=0.60, 95% CI=0.42-0.87). However, there was no relationship of C1236T polymorphism with ONFH susceptibility.

Analysis for *ABCB1* haplotypes and ONFH susceptibility

Results of linkage disequilibrium (LD) analysis indicated that the two SNPs of *ABCB1* C1236T and C3435T comprised four haplotypes (**Table 3**). Compared with C-C haplotype, T-T was a protective factor for ONFH (OR=0.45, 95% CI=0.23-0.87). However, C-T and

T-C haplotypes showed no effects on the pathogenesis of ONFH.

Discussion

In recent years, the incidence of ONFH is increasing year by year and the ONFH patients incline to the young man. The patients are likely to lose labor capacity and even the self-care ability without timely treatment, thus the disease consequently brings heavy burdens to the society and family. It is urgent to find out high-risk population of ONFH, which will contribute to early diagnosis and timely treatment for the patients.

Recently, it has been demonstrated that genetic polymorphisms could affect individual's susceptibility to cancers. *ABCB1* gene has been reported to relate with individual resistance to colchicine, vinblastine and doxorubicin [25]. Kim RB et al. found that *ABCB1* polymorphisms (C1236T, C3435T and G2677T) could affect the bioavailability of oral digoxins, fexofenadine and nelfinavir [26]. P-gp, encoded by *ABCB1* or *MDR1*, mainly expresses on the surface of

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enterocytes and brain capillary endothelial cells. As a transmembrane outflow pump, it is able to prevent foreign substances entering the cells and pump the drugs or poisonous substances out of the cells, supported by the energy transformation of ATP, thus influences the admittance of drugs [27]. Meanwhile, the studies showed that increased P-gp activity confers low risk for ONFH [20]. The expression of P-gp could be regulated by certain polymorphisms in *ABCB1* gene. Among them, the two synonymous SNPs of C1236T and C3435T were mostly studied.

In our case-control study, genotypes distribution of C1236T and C3435T polymorphisms in control group were in agreement with HWE, which indicated that the subjects were representative. TT genotype and T allele of *ABCB1* C3435T seemed to be inhibitors for ONFH. Moreover, T-T haplotype was also a protective factor for ONFH.

While, there were some limitations in the present research. The study only investigated two SNPs and more polymorphisms loci involved in the pathogenesis of ONFH may help us comprehensively understand the molecular mechanism of the disease. In addition, the sample size was relatively small and the investigations aiming at a larger scale of participants are required to provide a more precise estimation on the issue.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Weimin Jiang, Orthopedic Department of 1st Affiliated Hospital of Suzhou University, Suzhou, China. E-mail: zhang_890@126.com

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