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Original Article

Association of *ABCB1* polymorphisms with osteonecrosis of the femoral head risk

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Abstract: *Objectives*: The study was designed to examine the relationship between osteonecrosis of the femoral head (ONFH) risk and 1236 C>T (rs1128503) and 3435 C>T (rs1045642) polymorphisms of ATP-binding cassette sub-family B member 1 (*ABCB1*) gene. *Methods*: 120 healthy controls were frequency-matched with 100 ONFH patients by age and gender. Genotypes in 1236 C>T and 3435 C>T polymorphisms of *ABCB1* gene were detected in both groups by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Odds ratio (OR) and 95% confidence interval (CI) calculated by the chi-squared text were utilized to analyze the relationship between *ABCB1* polymorphisms and the ONFH susceptibility. Hardy-Weinberg equilibrium (HWE) was checked by the χ^2 text in the control group. *Results*: The genotypes distributions of the controls in the two polymorphisms were both consistent with HWE. There was no significant relevance between 1236 C>T polymorphism and ONFH risk (P>0.05). However, TT genotype in *ABCB1* 3435 C>T polymorphism remarkably decreased the risk of ONFH (OR=0.417, 95% CI=0.185-0.939) and T allele might be a protective factor for ONFH (OR=0.678, 95% CI=0.465-0.989). Based on haplotype analysis, T-C in 1236 C>T and 3435 C>T polymorphisms was 2.253 times risk for the development of ONFH compared with C-C haplotype (OR=2.253, 95% CI=1.063-4.773). *Conclusions*: The TT genotype and T allele of *ABCB1* 3435 C>T polymorphism might be the protective factors for ONFH. Further study with well-designed is needed in the future.

Keywords: *ABCB1,* osteonecrosis of the femoral head, polymorphism

Introduction

Osteonecrosis of the femoral head (ONFH), also known as ischemic necrosis of femoral head, is a common disease difficult to treat in orthopedics field [1]. It is featured from intermittent at first to persistent, muscle spasm, joint motion restriction, even severe disability [2, 3] and makes patients with ONFH be subjected to grave afflict and economic burden [4, 5]. The interruption or damage of blood supply in femoral head caused by the interactions among multiple factors leads to the death of constituents in bone cells and bone marrow [6-8]. So the development of ONFH is a consequence regulated by many factors, including gene and environment. It is very important to ascertain the pathogenesis of ONFH for the improvement of its diagnosis and treatment. In recent years, many researchers have proven the association between gene polymorphisms and the susceptibility to ONFH. For example, *eNOS*, *PON-1*, *PAI-1* genes polymorphisms can significantly increased the risk of ONFH [9-11].

ATP-binding cassette sub-family B member 1 (ABCB1), also called P-glycoprotein 1 (P-gp), is a protein encoded by *ABCB1* gene (*MDR1* or *CD243)* which is located on 7q21.1 in human chromosome and contains 28 exons [12]. P-gp can actively pump substrates entering cells, like chemicals and drugs out of cells to protect cells from the damage of poisons and metabolites [13, 14]. Some studies found that *ABCB1* gene polymorphisms were correlated with the metabolism and transformation of multiple

Table 1. Primer information of tested polymorphisms in *ABCB1*

SNP		1236 C>T	3435 C>T
Primer sequences	Forward	5'-TGAAGAGTTTCTGATGTTTT-3'	5'-TGTTTTCAGCTGCTTGATGG-3'
	Reverse	5'-CAAGAAAACATCAGAAACTC-3'	5'-AAGGCATGTATGTTGGCCTC-3'
Product length		294 bp	197 bp

Table 2. General data of the study objects

Clinical character	Case (n=100)	Control (n=120)	*P*
Age (mean ± SD)	49.25±3.15	51.19±4.89	0.527
Gender (male/female)	72/28	83/37	0.646
Body mass index (mean ± SD)	22.4±3.32	24.1±4.15	0.356

drugs and the human tolerance to drugs, and also had certain relationship with human susceptibility to diseases and with clinical manifestation [15, 16]. *ABCB1* gene polymorphisms have been reportedly firmed to be associated to the susceptibility of malignant tumors, such as glioma, leukemia and gastric cancer [17-20], but the study on its relationship with ONFH is insufficient.

In present study, two representative polymorphisms in *ABCB1* gene were chosen to examine their relationship with the susceptibility to ONFH in 100 patients with ONFH and 120 healthy controls, so as to provide theoretical guidance for the early diagnosis and treatment on ONFH.

Materials and methods

Study subjects

100 ONFH inpatients (72 males and 28 females) confirmed by clinical examination and X-ray detection were collected from Handan Xingtai Workers General Hospital of China Minmetals Corporation during November 2011 and April 2014 as the case group. Their age was ranged from 27-65. Among them, patients were excluded if they had hip trauma. 120 healthy controls (83 males and 37 females) frequency-matched by age and sex with the cases were recruited from people who made physical examination in the examination center of the hospital during the same period. They had good physical condition and no history of genetic diseases. Subjects in this study were all from the same region with similar backgrounds in life and environment, but were unrelated by blood. The study obtained the agreement from the Ethics Committee of Handan Xingtai Workers General Hospital of China Minmetals Corporation and the written informed consent of all subjects. The sample collection was operated in accordance with the national ethics criterion for human genome study.

Blood DNA extraction

5 ml peripheral blood was collected from every subject, and genome DNA of blood samples was extracted according to DNA extraction kit instruction. The extractive was quantified using ultraviolet spectrophotometer, adjusted to the appropriate concentration and stored at -20°C fridge.

Selection of polymorphisms in ABCB1 gene

Single nucleotide polymorphisms (SNPs) were chosen using human genome haplotype database. The conditions for selection were as follows: minor allele frequency (MAF) ≥0.05, Chinese Han population (CHP) and data from HapMap Data Rel 24/phas II Nov08, on NCBI B36 assembly, dbSNP b126. SNPs with $r^2 \geq 0.80$ were selected using linkage disequilibrium (LD) analysis. *ABCB1* gene polymorphisms were found that were closely related to ONFH susceptibility in previous articles, the study was determined to select two polymorphisms of 1236 C>T and C3435T in *ABCB1*.

Genotyping method

The genotype distribution was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in subjects. Primers were designed by Premier 5.0 software and the information is listed in **Table 1**. PCR reaction system was a total of 25 μl solution, including 2 μl primers (1 μl forward and 1 μl

Table 3. Genotype and allele distribution frequencies in *ABCB1* gene polymorphisms

Genotype/Allele	Case (n=100, %)	Control (n=120, %)	χ^2	*P*	OR (95% CI)
1236 C>T					
CC	33 (33.00)	41 (34.17)	-	-	1.00 (Ref.)
CT	47 (47.00)	63 (52.50)	0.063	0.802	0.927 (0.512-1.679)
TT	20 (32.00)	16 (13.33)	1.165	0.280	1.553 (0.697-3.461)
C	113 (56.50)	145 (60.42)	-	-	1.00 (Ref.)
T	87 (43.50)	95 (39.58)	0.690	0.406	1.175 (0.803-1.720)
3435 C>T					
CC	28 (28.00)	22 (18.33)			1.00 (Ref.)
CT	55 (55.00)	66 (55.00)	1.575	0.209	0.655 (0.337-1.271)
TT	17 (17.00)	32 (26.67)	4.531	0.033	0.417 (0.185-0.939)
C	111 (55.50)	110 (45.83)			1.00 (Ref.)
T	89 (44.50)	130 (54.17)	4.078	0.0430	0.678 (0.465-0.989)

Table 4. The haplotype analysis of 1236 C>T and 3435 C>T polymorphisms in *ABCB1*

Haplotypes	Cases (2 n=200, %)	Controls (2 n=240, %)	χ^2	*P*	OR (95% CI)
C-C	87 (43.50)	98 (40.83)			1.00 (Ref.)
C-T	26 (13.00)	47 (19.58)	2.769	0.096	0.623 (0.356-1.090)
T-C	24 (12.00)	12 (5.00)	4.650	0.031	2.253 (1.063-4.773)
T-T	63 (31.50)	83 (34.59)	0.495	0.482	0.855 (0.553-1.323)

reverse primers), 12.5 μl PCR Master Mix, 1 μl DNA plate and 9.5 μl of sterile water. The reaction procedure was as follows: initial degeneration at 95°C for 5 min; followed by 35 cycles of degeneration at 95°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 30 s; at last the final extension at 72°C for 10 min.

PCR amplified products were digested using relevant restriction endonuclease. 20 μl of total RFLP reaction mixture included 2 μl restriction endonuclease, 15 μl PCR products, 2 μl of 10× Buffer solution and 1 μl dd H_2O. The mixture was put in water bath at 37°C for 12 h. 4 μl digestion products were separated through 3% agarose gel electrophoresis and EB staining.

Statistical analysis

Statistical analysis was conducted using SPSS 18.0 software. χ^2 test was used to detect whether the goodness of fit of *ABCB1* gene conformed to Hardy-Weinberg Equilibrium (HWE) or not. Odds ratios (ORs) and 95% confidence intervals (CIs) were listed using cross table with $P<0.05$ representing statistical significance, which evaluated the association of *ABCB1* polymorphisms and ONFH risk.

Results

General information of the study objects

There were 220 samples in the study. The median age was 51.19±4.89 in controls while 49.25±3.15 in cases. The differences of age structure had no statistical significance between two groups ($P=0.527$), indicating the equilibrium of age distribution in two groups. Additionally, so as the gender structure between the cases and controls ($P=0.646$), which showed the balance of gender distributions in two groups and so body mass index (BMI) was ($P=0.356$). All results were listed in **Table 2**.

HWE test

The goodness of fit to the law for the genotypes distributions of *ABCB1* polymorphisms in controls was conformed to HWE ($P>0.05$), indicating the subjects we selected had good representativeness.

Association of genotypes in ABCB1 polymorphisms with the risk of ONFH

The polymorphism of *ABCB1* 1236 C>T was no significant associated with ONFH risk indepen-

dently. The TT genotype frequency of *ABCB1* 3435 C>T polymorphism significantly reduce the risk of ONFH compared with genotype CC (**Table 3**, OR=0.417, 95% CI=0.185-0.939). Similarly, T allele carriers might have the decreased risk for the occurrence of ONFH (OR= 0.678, 95%=0.465-0.989) and it was a protective factor.

Haplotype analysis

The linkage disequilibrium was found in *ABCB1* 1236 C>T and 3435 C>T polymorphisms and C-C, C-T, T-C, T-T haplotypes were analyzed. The frequency of haplotype T-C was higher in cases than the control group and significantly creased ONFH susceptibility compared with C-C haplotype. So haplotype T-C might be a risk factor for ONFH (**Table 4**, OR=2.253, 95% CI=1.063-4.773).

Discussion

ONFH shows an early age of onset and its morbidity is rising year by year. In recent years, its onset age tends to be younger, mainly in the man with 30-50 years old. If not treated in time, the patients will lose their labor capacities and even the abilities in everyday life, which brings heavy burden to society and family. At the moment, alcohol and corticosteroids have been universally acknowledged as the important risk factors for non-traumatic femoral head necrosis [21, 22], but its pathogenesis is not yet clear enough [23]. So genetic factors are concerned about the relevance with ONFH, especially gene polymorphism. Liu et al. made a survey in a Chinese population that CC genotype of *VEGF* -634G/C polymorphism significantly increased the risk of ONFH and it was a risk factor [24]. Lee et al. studied IVS7 +117 A>G polymorphism in *SREBF1* gene with ONFH risk in the Korean population and found that it was associated with the increased risk of ONFH [25]. Liu et al. also found that *MTHFR* 677 C/T polymorphism had the relationship with alcohol-induced ONFH [26].

ABCB1 gene has found over 50 SNPs, among which C-1236T and C-3435T are synonymous mutation while the others are non-synonymous mutation [27]. Previous studies have proven the association of *ABCB1* gene polymorphisms with the pathogenesis of multiple tumors. The study on hepatic carcinoma patients performed by Ren et al. found that a new polymorphism c4125 A>C in *ABCB1* gene had significant correlation with the cancer susceptibility [28]. Relevant research on Iran population also showed that 3435 C>T polymorphism in *ABCB1* gene was consistent with the occurrence frequency of gastric cancer [29]. There are few reports on the study of the relationship between *ABCB1* gene polymorphisms and ONFH susceptibility. The correlation analysis of gene polymorphisms with steroid-induced ONFH examined by Xue et al. on 662 Chinese using 3 SNPs of C-1236T, G-2677T/A and C-3435T manifested that C-3435T polymorphism in *ABCB1* gene was remarkably related to the susceptibility of steroid-induced ONFH [7].

In present study, through two representative SNPs in *ABCB1* gene, 1236 C>T and 3435 C>T, we explored the association between SNPs and the risk of ONFH. The polymorphism of 1236 C>T had no significantly association with ONFH susceptibility and it might have a role in the interaction with other polymorphisms. In 3435 C>T polymorphism of *ABCB1* gene, the distributions of TT genotype and T allele were 0.417 and 0.678 times higher in patients than in controls respectively, and they might serve as a protector for persons avoiding the trouble of ONFH.

Although we have obtained some achievements, but our results still were limited because of several conditions. Firstly, we only considered the single polymorphism and two polymorphisms in the same gene, the interaction of gene-environment was omitted. Secondly, the sample size was small and not enough to represent the relevance precisely. Thirdly, our results only showed the relationship between *ABCB1* polymorphisms with ONFH risk in several parts of China population.

In the conclusion, the polymorphism of 3435 C>T in *ABCB1* gene may be regulate the expression of *ABCB1* and modify the function, which affects the development of ONFH. Due to the small sample size, the study results still need to be repeatedly explored and verified on the rest of independent races and regions with more samples so as to provide scientific basis for the prevention and diagnosis of ONFH.

Disclosure of conflict of interest

None.

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