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Original Article

Association of ABCB1 C3435T and C1236T gene polymorphisms with the susceptibility to acute myeloid leukemia in a Chinese population

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Abstract: We conducted a case-control study to investigate the role of *ABCB1* C3435T (rs1045642) and C1236T (rs1128503) gene polymorphisms in the susceptibility to acute myeloid leukemia. The polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP) method was carried out to genotyping *ABCB1* C3435T and C1236T gene polymorphism. Statistical analysis was conducted using the SPSS 17.0 package (SPSS Inc., Chicago, IL, USA). Using Chi-square test, a statistically significant difference was found between acute myeloid leukemia patients and control subjects with respect to *ABCB1* C3435T (χ^2=8.04, P=0.02). By unconditional logistic regression analysis, we found that the TT genotype of *ABCB1* C3435T was associated with an increased risk of acute myeloid leukemia compared to the CC genotype (OR=2.24, 95% CI=1.22-4.12, P=0.01). In dominant model, the CT+TT genotype of *ABCB1* C3435T was correlated with a higher risk of acute myeloid leukemia than the CC genotype (OR=1.68, 95% CI=1.07-2.65, P=0.02). In the recessive model, the TT genotype of *ABCB1* C3435T contributed to the development of acute myeloid leukemia when compared to CC+CT genotype (OR=1.74, 95% CI=1.04-2.95). In our study, we suggested that the *ABCB1* C3435T genetic polymorphism could influence the risk of developing adult acute myeloid leukemia in co-dominant, dominant and recessive models.

Keywords: *ABCB1*, polymorphism, acute myeloid leukemia, Chinese population

Introduction

Acute myeloid leukemia is generally considered as a life threatening stem cell disease which is characterized by accumulation of myeloid blasts in the bone marrow. It is estimated that there are 352,000 new cases individuals with leukemia, and 265,000 individuals died from leukemia in 2012 worldwide [1]. There are two main types of acute leukemia, including acute lymphoblastic leukemia and acute myeloid leukemia [2]. The exact etiology of acute myeloid leukemia is not well understood currently. Previous studies have reported that many risk factors play an important role in the development of acute myeloid leukemia, including tobacco smoking, down syndrome, long-term exposing to benzene, family history of cancers and ionizing radiation [3, 4]. However, not all individuals would suffer from acute myeloid leukemia if they are exposure to the same potential risk factors of acute myeloid leukemia. Therefore, genetic factors may have a critical role in the pathogenesis of acute myeloid leukemia except environmental factors.

ATP-binding cassette sub-family B member 1 (*ABCB1*), named P-glycoprotein 1 (P-gp) or multidrug resistance protein 1 (*MDR1*), plays an important role in transporting a variety of molecules through extra-cellular and intra-cellular membranes [5]. The *ABCB1* gene is located on the chromosome 7q21.12, contains 29 exons and 28 introns, and encodes a membrane-associated protein of 1280 amino acids in length [6]. Up to now, 50 single nucleotide polymorphisms are found in *ABCB1* gene [7], and C3435T and C1236T are two common genetic polymorphisms in *ABCB1* gene. Previous studies have reported that *ABCB1* C3435T and

Table 1. The primers, amplification products and restriction digest products of *ABCB1* C3435T and C1236T

ABCB1	Primer sequences (5'-3')	Amplification products	Restriction digest products
C3435T	GATCTGTGAACTCTTGTTTTCA	244 bp	CC: 175 bp and 69 bp.
	GAAGAGAGACTTACATTAGGC		CT: 244 bp, 175 bp and 69 bp.
			TT: 244 bp.
C1236T	TCTTTGTCACTTTATCCAGC	502 bp	CC: 382 bp and 120 bp.
	TCTCACCATCCCCTCTGT		CT: 502 bp, 382 bp and 120 bp.
			TT: 502 bp.

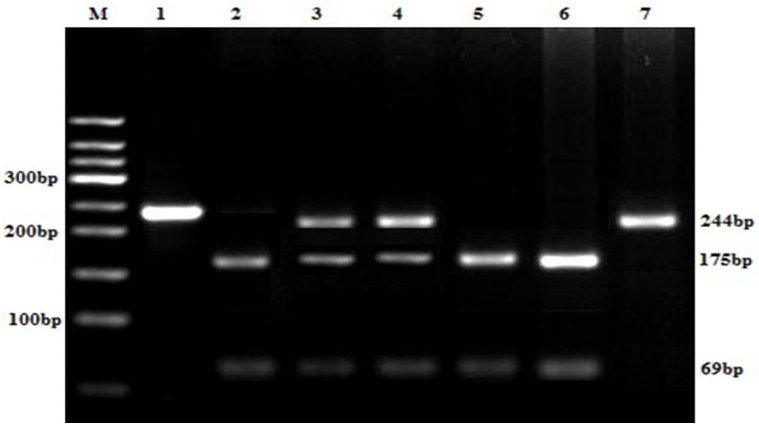

Figure 1. Agarose gel electrophoresis images for *ABCB1* C3435T. 2, 5 and 6 lanes: CC genotype; 1 and 7 lanes: TT genotype: 3 and 4 lanes: CT genotype.

C1236T may influence the immune responses and apoptosis and are associated with several kinds of diseases, such as colorectal cancer, breast cancer and hepatocellular carcinoma [8-11]. However, only three previous studies reported the association between *ABCB1* C3435T and C1236T polymorphisms and development of acute myeloid leukemia, and the results are conflicting [12-14]. Therefore, we conducted a case-control study to investigate the role of *ABCB1* C3435T (rs1045642) and C1236T (rs1128503) gene polymorphisms in the susceptibility to acute myeloid leukemia.

Material and methods

Subjects

Between January 2013 and December 2014, a total of 185 patients with acute myeloid leukemia were consecutively recruited from The First Affiliated Hospital of Zhengzhou University and the First Affiliated Hospital of Xinxiang Medical University. All the acute myeloid leukemia patients were confirmed by bone marrow histopathology. All the patients were confirmed to be without of other malignant tumors, history of myeloproliferative disease, history of no contact with treatment or drugs to cause leukemia and serious infection disease as well as serious kidney and liver diseases.

During the same period of time, a total of 225 control subjects were recruited from individuals of visiting outpatient clinics or receiving regular health examination in the First Affiliated Hospital of Xinxiang Medical University. All the control subjects were confirmed to be free of acute myeloid leukemia and other malignant tumors. The controls were free of hematological system disorders, and serious kidney and liver diseases.

The demographic and environmental characteristics were collected from medical records, including sex, tobacco smoking, exposure to benzene, age and family history of cancer. Written informed consents were obtained from acute myeloid leukemia patients and controls prior to participating into this study. Ethical approval for this study was obtained from the First Affiliated Hospital of Xinxiang Medical University.

Genotyping methods

Five ml blood sample was obtained from each participant, and blood samples were stored in tubes with 0.5 mg/ml EDTA and saved at -20°C until application. The polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP) method was carried out to genotyping *ABCB1* C3435T and

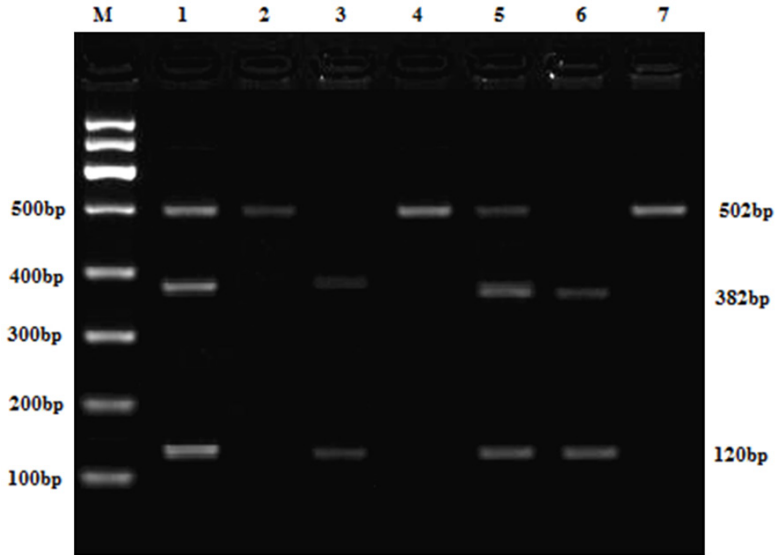

Figure 2. Agarose gel electrophoresis images for *ABCB1* C1236T. 3, 5 and 6 lanes: CC genotype; 1 and 5 lanes: CT genotype; 2 and 7 lanes: TT genotype.

Table 2. Demographic and environmental factors between acute myeloid leukemia patients and controls

Variables	Patients	%	Controls	%	Chi-square test	*P* value
Mean age, years	28.55±12.63		31.50±11.55		2.47	0.007
Sex						
Female	75	40.54	101	44.89		
Male	110	59.46	124	55.11	0.78	0.38
Tobacco smoking						
No	129	69.73	161	71.56		
Yes	56	30.27	64	28.44	0.16	0.69
Exposure to benzene						
No	149	80.54	197	87.56		
Yes	36	19.46	28	12.44	3.79	0.06
Family history of cancer						
No	162	87.57	206	91.56		
Yes	23	12.43	19	8.44	1.76	0.19

C1236T gene polymorphism. The primers, amplification products and restriction digest products of *ABCB1* C3435T and C1236T were shown in **Table 1**. The PCR reactions were conducted in a 20 μL volume, consisting of 3~4 μL genomic DNA, 0.2 μL Taq enzyme, 2.5 μL 10 × PCR mix, 2.0 μ 25 mol/L dNTP Mixture, 1.0 μL 0.5 μM forward primer and 1.0 μL 0.5 uM reverse primer. The PCR conditions were set as follows: 95°C for 5 min, 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s and a final extension step of 72°C for 10 min. The amplified products were digested with *Mbo*I and *Eco0109*I for *ABCB1* C3435T and C1236T, respectively. The PCR product was analyzed using 3% agarose gel electrophoresis to identify the purity and integrity, and the results were confirmed ultraviolet light (**Figures 1** and **2**).

Statistical analysis

Data were analyzed by independent sample t-test or Chi-square test. The distribution of genotypes in cases and controls was tested for deviation from Hardy-Weinberg equilibrium. We used the Chi-square test to examine differences in genotypic and allelic distribution between acute myeloid leukemia patients and controls. Association between ABCB1 C3435T and C1236T polymorphisms and acute myeloid leukemia was calculated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. Statistical analysis was conducted using the SPSS 17.0 package (SPSS Inc., Chicago, IL, USA). A *P*-value less than 0.05 were considered statistically significant.

Results

The demographic and environmental factors were compared between acute myeloid leukemia patients and controls using chi-square test (**Table 2**). All the acute myeloid leukemia patients were comparable in sex (chi-square=0.78, P=0.38), tobacco smoking (chi-square =0.16, P=0.69), exposure to benzene (chi-square=3.79, P=0.06) and family history of cancer (chi-square=1.76, P=0.19). There was statistically significant between acute myeloid leukemia

Table 3. Genotype distributions of *ABCB1* C3435T and C1236T polymorphisms between acute myeloid leukemia patients and control subjects

ABCB1	Patients	%	Controls	%	Chi-square test	*P* value	P for Hardy-Weinberg equilibrium	
C3435T							In patients	In controls
CC	47	25.41	82	36.44				
CT	93	50.27	108	48.00				
TT	45	24.32	35	15.56	8.04	0.02	0.94	0.95
C1236T								
CC	24	12.97	22	9.78				
CT	86	46.49	107	47.56				
TT	75	40.54	96	42.67	1.06	0.59	0.93	0.32

Table 4. Association between *ABCB1* C3435T and C1236T polymorphisms and development of acute myeloid leukemia

ABCB1	Patients	%	Controls	%	OR (95% CI)[1]	*P* value
C3435T						
Co-dominant						
CC	47	25.41	82	36.44	Ref. (1.0)	-
CT	93	50.27	108	48.00	1.50 (0.93-2.43)	0.08
TT	45	24.32	35	15.56	2.24 (1.22-4.12)	0.01
Dominant						
CC	47	25.41	82	36.44	Ref. (1.0)	-
CT+TT	138	74.59	143	63.56	1.68 (1.07-2.65)	0.02
Recessive						
CC+CT	140	75.68	190	84.44	Ref. (1.0)	-
TT	45	24.32	35	15.56	1.74 (1.04-2.95)	0.03
C1236T						
Co-dominant						
CC	24	12.97	22	9.78	Ref. (1.0)	-
CT	86	46.49	107	47.56	0.74 (0.36-1.48)	0.35
TT	75	40.54	96	42.67	0.71 (0.35-1.45)	0.31
Dominant						
CC	24	12.97	22	9.78	Ref. (1.0)	-
CT+TT	161	87.03	203	90.23	0.73 (0.37-1.41	0.31
Recessive						
CC+CT	110	59.46	129	57.34	Ref. (1.0)	-
TT	75	40.54	96	42.67	0.92 (0.61-1.39)	0.66

[1]Adjusted for age and sex.

patients and controls using Chi-square test (chi-square =2.47, P=0.007).

The genotype distribution of *ABCB1* C3435T and C1236T genes were shown in **Table 3**. In the acute myeloid leukemia patients, there were 47 (25.41%), 93 (50.27%) and 45 (24.32%) patients carried the CC, CT and TT genotypes of *ABCB1* C3435T, respectively, and there were 24 (12.97%), 86 (46.49%) and 75 (40.54%) carried the CC, CT and TT genotype of *ABCB1* C1236T, respectively. In the control subjects, there were 82 (36.44%), 108 (48.00%) and 35 (15.56%) carried the CC, CT and TT genotypes of *ABCB1* C3435T, respectively, and there were 22 (9.78%), 107 (47.56%) and 96 (42.67%) carried the CC, CT and TT genotype of *ABCB1* C1236T, respectively. Using Chi-square test, a statistically significant difference was found between acute myeloid leukemia patients and control subjects with respect to ABCB1 C3435T (χ^2=8.04, P=0.02). The genotype frequencies of ABCB1 C3435T and C1236T genes were in agreement with the Hardy-Weinberg equilibrium, using goodness-of-fit chi-square test.

By unconditional logistic regression analysis, we found that the TT genotype of *ABCB1* C3435T was associated with an increased risk of acute myeloid leukemia compared to the CC genotype (OR=2.24, 95% CI=1.22-4.12, P=0.01) (**Table 4**). In dominant model, the CT+TT genotype of *ABCB1* C3435T was correlated with a higher risk of acute myeloid leukemia than the CC genotype (OR=1.68, 95% CI=1.07-2.65, P=0.02). In the recessive model, the TT genotype of *ABCB1* C3435T contributed to the development of

acute myeloid leukemia when compared to CC+CT genotype (OR=1.74, 95% CI=1.04-2.95). However, the *ABCB1* C1236T did not show significant association with the risk of acute myeloid leukemia.

Discussion

In the present study, we investigated the role of *ABCB1* C3435T and C1236T genetic polymorphisms in the development of acute myeloid leukemia, and our findings indicated that the TT genotype of *ABCB1* C3435T gene affected the susceptibility to acute myeloid leukemia when compared to the CC genotype, and also the *ABCB1* C3435T gene polymorphisms was significantly with a higher risk of acute myeloid leukemia in dominant and recessive models.

ABCB1 gene encoding the expression of P-gp is driven ATP efflux pump, and *ABCB1* could pump out of the toxic substances which did not dissolve in water in the cell. *ABCB1* could increase the P-gp in the tumor cells, and it is hypothesis that the T allele could degrade the expression of *ABCB1* and decrease the function of P-gp [15]. Thus carcinogenic substances are easily accumulated in the tissue having ABCB1 expression, and the susceptibility to carcinogenesis is significantly increased.

Previous studies have reported that *ABCB1* gene polymorphism is associated with the development of cancers, including breast cancer, gastric cancer, colorectal cancer, hepatocellular carcinoma, prostate cancer and thyroid cancer [12, 16-19]. Gutierrez-Rubio et al. conducted a study with 248 women with breast cancer and 180 healthy controls, and indicated that *ABCB1* C3435T gene polymorphism may be a critical factor for developing breast cancer in premenopausal women [10]. Li et al. carried out a study with 645 hepatocellular cancer patients and 658 cancer-free control subjects, and they found that *ABCB1* 3751G>A genetic variants could influence the susceptibility to hepatocellular carcinoma [16]. Zhou et al. indicated that the *ABCB1* 3037A>C genetic polymorphism contributed to the susceptibility to gastric cancer in the Chinese Han population [17]. Shen et al. suggested that *ABCB1* 1465C>T genetic variation was associated with prostate cancer in Chinese men [18]. Ozdemir et al. conducted a study in a Turkish population, and they suggested that *ABCB1* C3435T genetic variation might influence the susceptibility to differentiated thyroid cancers [19]. However, some studies reported that the *ABCB1* gene polymorphism could not influence the risk of cancer [20, 21]. Zhang et al. reported that no significant association was found between *ABCB1* C3435T gene polymorphism and colorectal cancer risk [20]. Wu et al. suggested that the *ABCB1* C3435T genetic polymorphism is not associated with the susceptibility to gastric cancer [21].

For the association between *ABCB1* gene polymorphism and acute myeloid leukemia, only three studies reported their correlation and the results are inconclusive [12-14]. Ma et al. conducted a study with 178 acute leukemia patients and 150 healthy subjects in a Chinese population, and they reported that *ABCB1* C3435T polymorphism was significantly associated with the risk of developing acute leukemia [12]. Rao et al. conducted a study consisting of 143 acute lymphocytic leukemia patients and 249 control subjects in an Indian population, and they suggested that *ABCB1* C3435T TT genotype might influence risk of development of acute lympoblastic leukemia when compared with the CC genotype [13]. Jamroziak et al. conducted a study in a Polish population, and they found that *ABCB1* C3435T genetic variation could not influence the susceptibility to adult acute myeloid leukemia [14].

Two limitations of the present study should be taken into account. First, selection bias may have been present, as all patients and control subjects were recruited from one hospital. However, the *ABCB1* C3435T and C1236T polymorphism genotype distributions did not deviate from HWE, suggesting that the study subjects were representative of the general population. Second, other genes that may interact with ABCB1 were not included in our analysis. Thus, further studies with larger sample sizes are required to confirm the findings of our study.

In our study, we suggested that the *ABCB1* C3435T genetic polymorphism could influence the risk of developing adult acute myeloid leukemia in co-dominant, dominant and recessive models. Further large scale studies with more ethnicities are greatly needed to confirm the finding of our results.

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Disclosure of conflict of interest

None.

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