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Role of *ABCB1* C1236T, G2677T, and C3435T genetic polymorphisms in the development of acute leukemia in a Chinese population

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ABSTRACT. We carried out a case-control study to examine the relationship between the ATP-binding cassette subfamily B member 1 (*ABCB1*) gene polymorphisms C1236T, G2677T, and C3435T and risk of acute leukemia in a Chinese population. Between May 2013 and April 2015, we recruited 164 acute leukemia patients and 285 healthy controls, and determined polymorphism genotypes by polymerase chain reaction-restriction fragment length polymorphism. Using unconditional logistic regression analysis, we observed that in comparison to the wild-type sequence, the TT genotype [odds ratio (OR) = 2.15, 95% confidence interval (CI) = 1.12-4.10; P = 0.01] and the T allele (OR = 1.39, 95%CI = 1.05-1.86; P = 0.02) of *ABCB1* G2677T were associated with acute

leukemia susceptibility. The TT genotype (OR = 2.03, 95%CI = 1.11-3.69; P = 0.01) and the T allele (OR = 1.39, 95%CI = 1.05-1.85; P = 0.02) of the C3435T polymorphism also increased acute leukemia risk compared to the wild-type form. However, no significant relationship was established between the *ABCB1* C1236T variant and this disease. Our results suggest that the *ABCB1* G2677T and C3435T sequence variations may affect susceptibility to acute leukemia.

Key words: *ABCB1*; C3435T; C1236T; Polymorphism; Acute leukemia

INTRODUCTION

Acute leukemia is a serious malignancy of stem cells characterized by accumulation of myeloid blasts in bone marrow. An annual cancer report revealed that in 2012, 65,778 new cases of leukemia were diagnosed and 54,719 patients died as a result of this disease, accounting for 2.5% of all cancer deaths in that year worldwide (Ferlay et al., 2013). This disease is classified into two principal forms, namely, acute lymphoblastic leukemia and acute leukemia (Vardiman et al., 2009). In addition, genetic factors play an important role in susceptibility to acute leukemia, and prior research has shown many genes to be associated with its development, including those encoding endothelial protein C receptor, DNA repair enzymes, cytochrome P450 1A1, tumor protein p53, tet oncogene family member 2, and primary microRNA-34b/c (Besbes et al., 2015; Li et al., 2015; Lu et al., 2015; Ruan et al., 2015; Tong et al., 2015; Bănescu et al., 2016).

The *ABCB1* gene is located on chromosome 7q21.12 and encodes a protein of 1280 amino acids. G2677T (rs2032582) located in exon 21, C3435T (rs1045642) in exon 26, and C1236T (rs1128503) in exon 12 exhibit a certain degree of linkage (Rustemoglu et al., 2014; Saidijam et al., 2015). In recent years, several studies have evaluated the relationship between the *ABCB1* C1236T, G2677T, and C3435T polymorphisms and development of acute leukemia, but the results are conflicting (Urayama et al., 2007; Wang et al., 2007; Kreile et al., 2014; Pongstaporn et al., 2015). Therefore, we carried out a case-control study to assess the relationship between these *ABCB1* variants and acute leukemia risk in a Chinese population.

MATERIAL AND METHODS

Subjects

The present study comprised 164 acute leukemia patients and 285 healthy controls. The former were consecutively recruited from Qilu and Yantai Yuhuangding Hospitals between May 2013 and April 2015. Patients with a history of malignant cancer other than acute leukemia, autoimmune diseases, serious acute or chronic infectious diseases, end-stage liver or kidney diseases, or having previously used immunosuppressive agents were excluded from the study.

Healthy control subjects were selected from among individuals having visited the outpatient clinics of Qilu and Yantai Yuhuangding Hospitals over the same period. Those with a history of acute leukemia, any of the conditions mentioned above, or having previously used immunosuppressive drugs were excluded from the investigation.

Data regarding participants' demographic characteristics were collected using a questionnaire conducted by trained nurses in face-to-face interviews, and included age, gender, tobacco smoking, exposure to benzene, alkylating agents, and ionizing radiation, and family history of malignant cancer. Each subject signed an informed consent form prior to recruitment, and this study was approved by the Ethics Committees of Qilu and Yantai Yuhuangding Hospitals.

ABCB1 genotyping and direct sequencing

Each study subject provided a 5-mL blood sample for DNA extraction, which was stored in a tube containing 0.5 M ethylenediaminetetraacetic acid at 4°C until use. *ABCB1* C1236T, G2677T, and C3435T genotypes were determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism. The primers and restriction enzymes used are shown in Table 1. PCRs began with a denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 90 s, before a final extension at 72°C for 7 min. Digested PCR products were separated using 1.5% agarose gel electrophoresis and visualized under ultraviolet light.

Table 1. Primers and restriction enzymes for *ABCB1* C1236T, G2677T, and C3435T genotyping.

Polymorphism	Primers (5'-3')	Restriction enzyme
C1236T	TCTTTGTCACTTTATCCAGC (forward) TCTCACCATCCCCTCTGT (reverse)	*Eco*O109I
G2677T	TGCAGGCTATAGGTTCCAGG (forward) TTTAGTTTGACTCACCTTCCCG (reverse)	*Xba*I
C3435T	GATCTGTGAACTCTTGTTTTCA (forward) GAAGAGAGACTTACATTAGGC (reverse)	*Mbo*I

Statistical analysis

Differences between acute leukemia patients and control subjects in terms of demographic and lifestyle factors and *ABCB1* polymorphism genotypes were assessed using the Pearson chi-square test or the Student *t*-test. Conformance of *ABCB1* C1236T, G2677T, and C3435T genotype frequencies to Hardy-Weinberg equilibrium (HWE) was established using the Pearson chi-square test. Multivariate logistic regression analyses were carried out to estimate the relationship between these genetic variants and acute leukemia risk, employing odds ratios (ORs) and their 95% confidence intervals (CIs). Statistical analysis was conducted with SPSS 20.0 (IBM Corp., Armonk, NY, USA), and a P value <0.05 was considered to signify a significant difference.

RESULTS

In comparison with the control subjects, acute leukemia patients were younger (chi-square = 2.52, P = 0.01; Table 2). However, the two groups were comparable with respect to gender (chi-square = 0.18, P = 0.67), tobacco smoking (chi-square = 0.25, P = 0.62), exposure to benzene or alkylating agents (chi-square = 1.53, P = 0.22), exposure to ionizing radiation (chi-square = 2.39, P = 0.12), and family history of malignant cancer (chi-square = 0.85, P = 0.36).

Table 2. Demographic and environmental circumstances of acute leukemia patients and control subjects.

Variable	Patients (N = 164)	%	Controls (N = 285)	%	Chi-square	P
Mean age (years)	28.55 ± 12.63		31.50 ± 11.55		2.52	0.01
Gender						
Female	68	41.46	124	43.51		
Male	96	58.54	161	56.49	0.18	0.67
Tobacco smoking						
No	122	74.39	218	76.49		
Yes	42	25.61	67	23.51	0.25	0.62
Exposure to benzene or alkylating agents						
No	137	83.54	250	87.72		
Yes	27	16.46	35	12.28	1.53	0.22
Exposure to ionizing radiation						
No	153	93.29	275	96.49		
Yes	11	6.71	10	3.51	2.39	0.12
Family history of malignant cancer						
No	142	86.59	255	89.47		
Yes	22	13.41	30	10.53	0.85	0.36

ABCB1 C1236T, G2677T, and C3435T genotype distributions are summarized in Table 3. Using the Pearson chi-square test, significant differences were evident in the frequencies of *ABCB1* G2677T (chi-square = 6.31, P = 0.04) and C3435T (chi-square = 6.35, P = 0.04) genotypes between the patient and control groups. However, no such trend was observed in relation to the C1236T polymorphism (chi-square = 0.22, P = 0.90). None of the *ABCB1* variants demonstrated deviation from HWE in either the patient or control group.

Table 3. *ABCB1* C1236T, G2677T, and C3435T polymorphism genotype frequencies among acute leukemia patients and control subjects.

ABCB1	Patients (N = 164)	%	Controls (N = 285)	%	Chi-square	P	P for Hardy-Weinberg equilibrium	
							Patients	Controls
C1236T								
CC	21	12.80	33	11.58				
CT	78	47.56	134	47.02				
TT	65	39.63	118	41.40	0.22	0.90	0.75	0.59
G2677T								
GG	50	30.49	111	38.94				
GT	84	51.22	143	50.18				
TT	30	18.29	31	10.88	6.31	0.04	0.61	0.13
C3435T								
CC	47	28.66	103	36.14				
CT	79	48.17	141	49.47				
TT	38	23.17	41	14.39	6.35	0.04	0.67	0.51

Use of unconditional logistic regression analysis revealed that the TT genotype (OR = 2.15, 95%CI = 1.12-4.10; P = 0.01) and the T allele (OR = 1.39, 95%CI = 1.05-1.86; P = 0.02) of *ABCB1* G2677T were associated with acute leukemia, in comparison to the wild-type sequence (Table 4). Furthermore, the TT genotype (OR = 2.03, 95%CI = 1.11-3.69; P = 0.01) and the T allele (OR = 1.39, 95%CI = 1.05-1.85; P = 0.02) of *ABCB1* C3435T increased risk of this disease, also when compared to the wild type. However, no significant relationship was apparent between the *ABCB1* C1236T variant and susceptibility to acute leukemia.

Table 4. Association between *ABCB1* C1236T, G2677T, and C3435T polymorphisms and acute leukemia.

ABCB1	Patients (N = 164)	%	Controls (N = 285)	%	OR (95%CI)[1]	P
C1236T						
CC	21	12.80	33	11.58	1.0 (Ref.)	-
CT	78	47.56	134	47.02	0.89 (0.46-1.74)	0.70
TT	65	39.63	118	41.40	0.84 (0.43-1.67)	0.58
Allele						
C	120	36.59	200	35.09	1.0 (Ref.)	-
T	208	63.41	370	64.91	0.94 (0.70-1.26)	0.65
G2677T						
GG	50	30.49	111	38.94	1.0 (Ref.)	-
GT	84	51.22	143	50.18	1.30 (0.83-2.05)	0.22
TT	30	18.29	31	10.88	2.15 (1.12-4.10)	0.01
Allele						
G	184	56.10	365	64.04	1.0 (Ref.)	-
T	144	43.90	205	35.96	1.39 (1.05-1.86)	0.02
C3435T						
CC	47	28.66	103	36.14	1.0 (Ref.)	-
CT	79	48.17	141	49.47	1.23 (0.77-1.96)	0.36
TT	38	23.17	41	14.39	2.03 (1.11-3.69)	0.01
Allele						
C	173	52.74	347	60.88	1.0 (Ref.)	-
T	155	47.26	223	39.12	1.39 (1.05-1.85)	0.02

[1]Adjusted for age and gender. OR = odds ratio, CI = confidence interval, Ref. = reference.

DISCUSSION

In the present study, we explored the relationship between genetic variations in *ABCB1* and risk of acute leukemia, revealing that the G2677T and C3435T variants may influence susceptibility to this condition in the Chinese population.

Recently, many investigations have reported that *ABCB1* genetic mutations have a substantial influence on immune responses and apoptosis, and are associated with the development of various malignancies, including colorectal, breast, and lung cancer, hepatocellular carcinoma, and non-Hodgkin lymphoma (Kim et al., 2014; Wan et al., 2014; Gutierrez-Rubio et al., 2015; Kopp et al., 2015; Zhu et al., 2015). For instance, Kim et al. (2014) carried out a population-based study of 694 non-Hodgkin lymphoma patients and 1700 controls, reporting that the *ABCB1* C3435T polymorphism may contribute to the risk of developing this disease in Koreans. In addition, Wan et al. (2014) demonstrated that the *ABCB1* A1564T sequence variation in this gene may affect susceptibility to hepatocellular carcinoma. Kopp et al. (2015) conducted an investigation involving 1010 colorectal cancer patients and 1829 control subjects, the results of which suggested that *ABCB1* C3435T is involved in the biological mechanism underlying carcinogenesis in the colorectum. Similarly, Gutierrez-Rubio et al. (2015) revealed that the T allele of this variant significantly increases breast cancer risk; however, Zhu et al. (2015) found no significant relationship between this polymorphism and lung cancer.

Several reports concerning the relationship between *ABCB1* sequence variants and acute leukemia have been published, but have reached conflicting conclusions (Kreile et al., 2014; Pongstaporn et al., 2015). Two studies found that the C1236T and C3435T polymorphisms might influence susceptibility to acute leukemia in Indian, Thai, and Chinese populations (Rao et al., 2010; Pongstaporn et al., 2015). However, three other analyses have reported contrasting results. Kreile et al. (2014) failed to establish significant relationships between *ABCB1* G2677T and C3435T and this disease, while an association with the latter

polymorphism was also ruled out by Jamroziak et al. (2006) and Leal-Ugarte et al. (2008) using data from Polish and Mexican populations. Such inconsistent results may be attributed to differences in populations, selection of study subjects, and sample sizes.

Two limitations to this study should be recognized. First, gene-gene and gene-environment interactions were not assessed in our analysis. Second, the relatively small sample size used may have reduced our investigation's statistical power, masking differences between the two study groups.

In conclusion, we suggest that the *ABCB1* G2677T and C3435T polymorphisms affect the risk of developing acute leukemia. Further large-scale studies involving additional ethnic groups are needed to confirm our findings.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Bănescu C, Iancu M, Trifa AP, Dobreanu M, et al. (2016). Influence of *XPC, XPD, XPF*, and *XPG* gene polymorphisms on the risk and the outcome of acute myeloid leukemia in a Romanian population. *Tumour Biol.* [Epub ahead of print]. http://dx.doi.org/10.1007/s13277-016-4815-6

Besbes S, Althawadi H, Alfarsi H, Mirshahi S, et al. (2015). Endothelial protein C receptor gene 6936A/G single-nucleotide polymorphism as a possible biomarker of thrombotic risk in acute myeloid leukemia. *Mol. Clin. Oncol.* 3: 1280-1284.

Ferlay J, Soerjomataram I, Ervik M, Dikshit R, et al. (2013). GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. International Agency for Research on Cancer, Lyon, France. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx. Accessed October 10, 2015.

Gutierrez-Rubio SA, Quintero-Ramos A, Durán-Cárdenas A, Franco-Topete RA, et al. (2015). 1236 C/T and 3435 C/T polymorphisms of the *ABCB1* gene in Mexican breast cancer patients. *Genet. Mol. Res.* 14: 1250-1259. http://dx.doi.org/10.4238/2015.February.13.3

Jamroziak K, Balcerczak E, Cebula B, Janus A, et al. (2006). No influence of 3435C>T *ABCB1* (*MDR1*) gene polymorphism on risk of adult acute myeloid leukemia and P-glycoprotein expression in blast cells. *Ther. Drug Monit.* 28: 707-711. http://dx.doi.org/10.1097/01.ftd.0000245770.75097.3f

Kim HN, Kim NY, Yu L, Kim YK, et al. (2014). Polymorphisms in DNA repair genes and *MDR1* and the risk for non-Hodgkin lymphoma. *Int. J. Mol. Sci.* 15: 6703-6716. http://dx.doi.org/10.3390/ijms15046703

Kopp TI, Andersen V, Tjonneland A and Vogel U (2015). Polymorphisms in ATP-binding cassette transporter genes and interaction with diet and life style factors in relation to colorectal cancer in a Danish prospective case-cohort study. *Scand. J. Gastroenterol.* 50: 1469-1481. http://dx.doi.org/10.3109/00365521.2015.1056224

Kreile M, Rots D, Piekuse L, Cebura E, et al. (2014). Lack of association between polymorphisms in genes *MTHFR* and *MDR1* with risk of childhood acute lymphoblastic leukemia. *Asian Pac. J. Cancer Prev.* 15: 9707-9711. http://dx.doi.org/10.7314/APJCP.2014.15.22.9707

Leal-Ugarte E, Gutiérrez-Angulo M, Macías-Gómez NM, Peralta-Leal V, et al. (2008). *MDR1* C3435T polymorphism in Mexican children with acute lymphoblastic leukemia and in healthy individuals. *Hum. Biol.* 80: 449-455. http://dx.doi.org/10.3378/1534-6617-80.4.449

Li MJ, Yang YL, Lee NC, Jou ST, et al. (2015). Tet oncogene family member 2 gene alterations in childhood acute myeloid leukemia. *J. Formos. Med. Assoc.* pii: S0929-6646(15)00299-5 [Epub ahead of print].

Lu J, Zhao Q, Zhai YJ, He HR, et al. (2015). Genetic polymorphisms of *CYP1A1* and risk of leukemia: a meta-analysis.

Onco Targets Ther. 8: 2883-2902. http://dx.doi.org/10.2147/OTT.S92259

Pongstaporn W, Pakakasama S, Chaksangchaichote P, Pongtheerat T, et al. (2015). *MDR1* C3435T and C1236T polymorphisms: association with high-risk childhood acute lymphoblastic leukemia. *Asian Pac. J. Cancer Prev.* 16: 2839-2843. http://dx.doi.org/10.7314/APJCP.2015.16.7.2839

Rao DN, Anuradha C, Vishnupriya S, Sailaja K, et al. (2010). Association of an MDR1 gene (C3435T) polymorphism with acute leukemia in India. *Asian Pac. J. Cancer Prev.* 11: 1063-1066.

Ruan XL, Li S, Geng P, Zeng XT, et al. (2015). Association between *TP53* gene codon 72 polymorphism and acute myeloid leukemia susceptibility: evidence based on a meta-analysis. *Med. Sci. Monit.* 21: 3048-3053. http://dx.doi.org/10.12659/MSM.894625

Rustemoglu A, Gumus-Akay G, Karakus N, Yigit S, et al. (2014). Association analysis of three *ABCB1* (*MDR1*) gene variants (C1236T, G2677A/T and C3435T) and their genotype/haplotype combinations with the familial Mediterranean fever. *Xenobiotica* 44: 933-940. http://dx.doi.org/10.3109/00498254.2014.915071

Saidijam M, Mahjub H, Shabab N and Yadegarazari R (2015). Simultaneous analysis of multidrug resistance 1 (*MDR1*) C3435T, G2677T/A, and C1236T genotypes in Hamadan City population, West of Iran. *Iran. Biomed. J.* 19: 57-62.

Tong N, Chu H, Wang M, Xue Y, et al. (2015). Pri-miR-34b/c rs4938723 polymorphism contributes to acute lymphoblastic leukemia susceptibility in Chinese children. *Leuk. Lymphoma* Nov 16: 1-6 [Epub ahead of print].

Urayama KY, Wiencke JK, Buffler PA, Chokkalingam AP, et al. (2007). *MDR1* gene variants, indoor insecticide exposure, and the risk of childhood acute lymphoblastic leukemia. *Cancer Epidemiol. Biomarkers Prev.* 16: 1172-1177. http://dx.doi.org/10.1158/1055-9965.EPI-07-0007

Vardiman JW, Thiele J, Arber DA, Brunning RD, et al. (2009). The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 114: 937-951. http://dx.doi.org/10.1182/blood-2009-03-209262

Wan YY, Wang XW, Hui HX and Wan L (2014). Association between the c.1564A>T genetic polymorphism of the *MDR1* gene and hepatocellular carcinoma in Chinese population. *Genet. Mol. Res.* 13: 6820-6826. http://dx.doi.org/10.4238/2014.August.29.3

Wang D, Ke XY, Wang J, Xu F, et al. (2007). Correlation between *MDR1* genetic polymorphism and prognosis in acute myeloid leukemia. *Zhonghua Yi Xue Za Zhi* 87: 1384-1388.

Zhu DQ, Zou Q, Hu CH, Su JL, et al. (2015). *XRCC1* genetic polymorphism acts a potential biomarker for lung cancer. *Tumour Biol.* 36: 3745-3750. http://dx.doi.org/10.1007/s13277-014-3014-6