RESEARCH ARTICLE

MDR1 C3435T and C1236T Polymorphisms: Association with High-risk Childhood Acute Lymphoblastic Leukemia

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Abstract

Background: *MDR1*, one of the most important drug-transporter genes, encodes P- glycoprotein (P-gp)-a transporter involved in protecting against xenobiotics and multi-drug resistance. The significance of the genetic background in childhood acute lymphoblastic leukemia (ALL) is not well understood. Materials and Methods: To evaluate whether C3435T and C1236T *MDR1* polymorphisms are associated with the occurrence and outcome of ALL, 208 children with ALL (median age 5.0 yr) and 101 healthy Thai children were studied by polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) assay. Results: C3435T and C1236T *MDR1* polymorphism are significantly associated with the high-risk group (OR= 2.6, 95%CI =1.164-5.808; P =0.028 and OR= 2.231, 95%CI =1.068-4.659; p=0.047, respectively), indicating that both may be candidates for molecular markers in the high-risk group of ALL.

Keywords: Acute lymphoblastic leukemia - *MDR1* polymorphism - high-risk group - Thailand

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy worldwide. Thanks to improvements in the treatment of childhood ALL, 30% of cases are cured. However, 80% of patients remain in the remission stage for 10 years, and 20% of children suffer recurrence, making the final cure rate 25-40% (Claus et al., 2012). The effects of various prognostic factors on the clinical outcome of ALL have been investigated with improvements in treatment, however, increased use of more intensive therapy has led to the emergence of new adverse sequelae, especially in high-risk cases (Pui et al., 2004).

Inherited susceptibility and specific environmental exposure are supposed to play a major role in the pathogenesis of ALL (Martin et al., 2007). Moreover, drug effects may be different in the presence of alterations of genes involved in drug metabolism. Functional polymorphism of genes that encode proteins important for the metabolism of anti-cancer drugs (such as the thiopurine methyl transferase gene, or the reduced folate carrier gene) influence the outcome of chemotherapy in ALL (McLeod et al., 2000; Lavedrier et al., 20002). Drug transporters are the major proteins crucial for drug metabolism, and the *MDR1* gene, encoding for P-glycoprotein (P-gp), is the most important of these drug transporters. P-gp is a 170 kd member of the adenosine triphosphate-binding cassette (ABC) superfamily of membrane transporters (Kerb, 2006). The most important physiologic role of P-gp is in protecting the organism against toxic xenobiotics associated with mutagenic activity. MDR is a major challenge to effective chemotherapeutic intervention against cancer. Recently, the mechanism underlying this phenomenon have been reviewed thoroughly. (Ullah, 2008). Likewise, P-glycoprotein expression in tumor cells is associated with the multi-drug resistance phenotype in some hematological malignancies (Jamroziak and Rodak, 2004).

Several studies on the effects of C1236T (rs128503), C3435T (rs1045642), and G2677T/A (rs2032582) SNPs on *MDR1* expression and function in tissues have been conducted in different populations (Kim et al., 2001; Taheri et al., 2010; Samanian et al., 2011). In addition, several lines of evidence have shown a significant correlation between C3435T polymorphism and risk factors or clinical outcomes of human cancer, such as hematologic malignancy (Sheng et al., 2012), breast and renal cancers (Wang et al., 2012), lung cancer (Wei et al., 2012), colorectal cancer (He et al., 2013), thyroid cancer (Ozmedir et al., 2013). Moreover, in Chinese non small lung cancer patients, it was shown that CC genotype of C3435T polymorphism is significantly associated with response rate of platinum-based chemotherapy (Yan et

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al., 2011). However, some evidence have shown there is no significant correlation between C3435T polymorphism and risk factors of cancer, such as gastric cancer (Wu et al., 2014) and chronic lymphocytic leukemia (Dong et al., 2011). The conflict results may be due to ethnic sampling and number of cases.

In this study, the association between C3435T and C1236T polymorphisms of the *MDR1* gene and the clinical characteristics of ALL were observed. The results may help develop new candidate molecular markers for ALL.

Materials and Methods

Specimens

In this retrospective study, 207 ALL patients (86 females and 121 males) with a median age of 5 years (range 1-14 years), and 101 healthy Thai children (45 females and 56 males) with a median age of 12 years (range 2-13 years), were assessed by the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. This study was approved by the Ethics Committee, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. Patients were classified by risk-based assignment protocol (Smith et al., 1996). In general, risk was defined by initial white blood cell count, age at diagnosis, French American British morphology, and lymphomatous disease status. Moreover, clinical data including age at diagnosis, sex, immunophenotype, and chromosome abnormalities were studied retrospectively.

DNA isolation

Genomic DNA was isolated from 3ml fresh EDTA blood by proteinase K digestion and salting out method (Miler et al., 1988) with some modification. Red blood cells were briefly lysed with 100% Triton X-100, and the white blood cell pellets were incubated in lysis buffer (10 mM Tris HCl, pH8.0, 400 mM NaCl, 2mM EDTA), 200 µL of 10% SDS and 10mg/ml proteinase K at 60°C for 3 hours. The solution was mixed with 6M NaCl, then shaken and centrifuged at 10,000g at 4°C for 10min. DNA was precipitated by absolute ethanol and washed three times with 70% cold ethanol. The DNA pellet was dissolved in TE buffer and kept at -20°C prior to use.

Genotyping of MDR1 C3435T polymorphism

Genotyping of *MDR1* C3435T polymorphism of ALL patients and controls was performed by PCR-RFLP assay, as described previously (Jamroziak et al., 2004). The C3435T polymorphism of *MDR1* was detected after PCR amplification using forward primer 5' TTG ATG GCA AAG AAA TAA AGC 3' and reverse primer 5' CTT ACA TTA GGC AGT GAC TCG 3'. DNA amplification was carried out in 50µL reaction mixture containing PCR buffer (10mM Tris-HCl, pH 9.0 50mM KCl, 1.5mM MgCl2) 200 µM of each dNTP, 1 unit of Taq DNA polymerase (Pharmacia, Biotech, USA), 20µM of each primer and 100ng of genomic DNA. Thermal cycling was performed in a Gene Amp PCR System 9700 (Perkin Elmer, USA) for 30 cycles; each consisting of denaturation at 94°C for 90s, annealing at 56°C for 60s and extension at 72°C for 90s. PCR amplicons were digested by MboI at 37°C for 16 hours, electrophoresed on 2% agarose gel, stained with ethidium bromide, and visualized under UV light. The digested product showed 3 different patterns; wild type homozygote(C/C), demonstrating 130 and 76 bp fragments, polymorphic homozygote (T/T) with 206 bp fragments and C/T heterozygote with 206, 130, and 76 bp fragments.

Genotyping of MDR1 C1236T polymorphism

Genotyping of *MDR1* C1236T polymorphism of ALL patients and controls was performed by PCR-RFLP assay with modification (Ryu et al., 2006). The C1236T polymorphism of *MDR1* was detected after PCR amplification using forward primer 5' TGT GTC TGT GAA TTG CCT TGA 3' and reverse primer 5' ATC TCA CCA TCC CCT CTG TG 3'. DNA amplification was carried out in 50µL of reaction mixture containing PCR buffer (10mM Tris-HCl, pH 9.0 50mM KCl, 1.5mM MgCl2), 200µM of each dNTP, 1 unit of Taq DNA polymerase (Pharmacia, Biotech, USA), 20µM of each primer and 100ng of genomic DNA. Thermal cycling was performed in a Gene Amp PCR System 9700 for 40 cycles; each consisted of denaturation at 94°C for 60s, annealing at 56°C for 60s, extension at 72°C for 60s, and final extension 72°C for 5min. PCR amplicons were digested by HaeIII at 37°C for 16 hours, electrophoresed on 4% NuSieve Agarose SFRTM, stained with ethidium bromide, and visualized under UV light. The digested product showed 3 different patterns; wild type homozygote(C/C), demonstrating 93 and 87bp fragments, polymorphic homozygote (T/T) showing 87, 58, and 35 bp fragments, and C/T heterozygote, showing 93, 87, 58, and 35 bp fragments.

Statistical analysis

Differences in genotypes between cases and controls were determined by Chi-square test. Crude odds ratios (OR) and 95% confidence intervals (CI) were also calculated. The association between C3435T and C1236T polymorphism of *MDR1* and prognostic factors for ALL, e.g. age at diagnosis, sex, gender, immunophenotype and risk group, were calculated by binary logistic regression analysis using the SPSS version 11.5 program (SPSS Inc., Chicago, USA).

Results

The association between C3435T and C1236T of MDR1 polymorphisms and ALL susceptibility

The allele frequencies for C3435T and C1236T of *MDR1* polymorphisms were determined in the childhood ALL and control groups. The allele frequency of C3435T *MDR1* polymorphism in the controls was 0.43, compared with 0.41 in ALL patients, and the allele frequency of C1236T *MDR1* polymorphism in the controls was 0.43, compared with 0.46 in ALL patients. All allele frequencies were in agreement with the Hardy–Weinberg equilibrium. There was no statistically significant correlation between the allele frequency of either C3435T *MDR1* polymorphism or C1236T *MDR1* polymorphism and ALL susceptibility (OR=0.811, 95%CI=0.483-1.363, p=0.510)

and (OR=1.3, 95%CI=0.7417-2.4680, p=0.6898), respectively. (Table1).

The association between C3435T and C1236T of MDR1 polymorphisms and high risk ALL

With modern chemotherapy, ALL patients can be classified as high-risk (white blood cell count >50,000 cell/μl, age > 10 years, or with T-cell immunophenotype), and low-risk (white blood cell count <50,000/μl, or age <10 years). This study indicates that the high-risk ALL group was significantly associated with C3435T (OR= 2.6, 95%CI =1.164-5.808; p=0.028) (Table 2) and C1236T *MDR1* polymorphism (p=0.028, OR=2.231, 95%CI =1.068-4.659; p=0.047) (Table3).

Table 1. Distribution of C3435T and C1236T MDR1 Genotypes in ALL, Compared with Controls

Genotypes	ALL (%)	Controls (%)	OR (95%CI)	P-value
MDR1 (exon26/3435)				
C/C	69 (33.17)	29 (28.71)	1.00(Reference)	
C/T	106 (50.96)	58 (57.43)	0.77(0.45-1.32)	0.41
T/T	33 (15.87)	14 (13.86)	0.99(0.46-2.12)	0.86
C/T+T/T	139 (66.83)	72 (71.28)	0.81(0.48-1.36)	0.51
Allele frequency				
C3435	0.59	0.57		
	0.41	0.43		
MDR1 (exon12/1236)				
C/C	34 (17.43)	22 (22.22)	1.00(Reference)	
C/T	141 (72.31)	68 (68.69)	1.34(0.73-2.47)	0.43
T/T	20 (10.26)	9 (9.09)	1.44(0.55-3.72)	0.61
C/T+T/T	161 (82.56)	71 (71.72)	1.35(0.74-2.47)	0.69
Allele frequency				
C1236	0.54	0.57		
T1236	0.46	0.43		

Table 2. Demographic Data of ALL Patients of C3435T MDR1 Genotypes

Demographic	Total patients	MDR1		
data	n (%)	CC	C/T or T/T	p value
Sex				
Male	121	36 (29.75)	85 (70.25)	0.253
Female	86	33 (38.37)	53 (61.63)	
Age at diagnosis				
1-10 years	136	49 (36.03)	87 (63.97)	0.149
>10 years	51	12 (23.53)	39 (76.47)	
Risk classification				
Standard risk	136	51 (37.5)	85 (62.5)	0.028*
High Risk	48	9 (18.75)	39 (81.25)	
Immunophenotype				
Non T cell	168	55 (32.74)	113 (67.26)	0.306
T cell	21	4 (19.05)	17 (80.95)	

Table 3. Demographic Data of ALL Patients of C1236T MDR1 Genotypes

Demographic	Total patients	MDR1		
data	n (%)	CC	C/T or T/T	p value
Sex				
Male	117	43 (36.75)	74 (63.25)	0.127
Female	80	39 (48.75)	41 (51.25)	
Age at diagnosis				
1-10 years	136	59 (43.38)	77 (56.62)	0.767
>10 years	51	21 (41.18)	30 (58.82)	
Risk classification				
Standard risk	136	58 (42.65)	78 (57.35)	0.047*
High Risk	48	12 (25)	36 (75)	
Immunophenotype				
Non T cell	157	66 (42.04)	91 (57.96)	0.784
T cell	22	8 (36.36)	14 (63.64)	

Clinical characteristic of high risk ALL

The correlation between the clinical characteristics of the ALL patients in the high-risk group with the C1236T

Table 4. Clinical Data of ALL in the High Risk Group and MDR1 Gene Polymorphism (n=48)

No.	Age >10 years	WBC >50,000/mL	Immuno-phenotype	C3435T	C1236T
1	yes	yes	Pre-B	C/T	C/C
2	No	No	T-cell	C/C	C/T
3	No	No	T-cell	C/T	C/T
4	yes	yes	Early-PreB	C/T	C/T
5	yes	yes	Pre-B	C/T	C/T
6*	yes	No	Pre-B	C/T	C/T
7	yes	yes	Pre-B	C/T	C/C
8	yes	No	Pre-B	T/T	C/T
9	yes	yes	T-cell	C/T	C/T
10	yes	No	Pre-B	T/T	C/T
11	yes	No	Pre-B	C/T	C/T
12	yes	No	Pre-B	C/T	C/T
13	yes	No	Pre-B	T/T	C/T
14	No	yes	T-cell	C/T	C/C
15*	yes	No	Pre-B	C/C	C/T
16	yes	yes	Pre-B	C/T	C/C
17	No	No	T-cell	C/T	C/T
18	No	yes	T-cell	C/T	C/T
19	No	yes	Pre-B	C/T	C/C
20	No	yes	T-cell	C/T	C/C
21	yes	yes	T-cell	C/T	C/T
22	yes	yes	Pre-B	C/T	C/C
23	No	yes	Pre-B	T/T	C/C
24*	yes	No	Pre-B	C/T	C/T
25	No	yes	Pre-B	C/T	C/T
26	yes	No	T-cell	C/T	C/T
27	yes	yes	T-cell	C/T	C/T
28	yes	No	T-cell	T/T	C/C
29	yes	yes	B-cell	C/T	C/T
30	yes	No	Pre-B	C/T	C/T
31	yes	No	T-cell	C/T	C/T
32	yes	No	Pre-B	C/C	C/C
33	yes	No	Pre-B	C/T	C/T
34	yes	No	T-cell	T/T	C/T
35	No	No	Early-PreB	C/C	C/T
36	No	No	Early-PreB	C/C	C/T
37	yes	yes	T-cell	C/T	C/T
38	yes	No	Early-PreB	C/C	C/C
39	yes	No	Mixed	C/T	C/C
40	yes	No	Early-PreB	T/T	C/T
41*	No	No	Early-PreB	C/T	T/T
42*	No	No	Pre-B	C/T	C/T
43*	No	No	B-cell	T/T	T/T
44	No	No	T-cell	T/T	C/T
45	No	No	Early-PreB	C/C	C/C
46*	No	No	Pre-B	C/C	C/T
47*	yes	yes	Early-PreB	C/C	C/T
48*	No	No	Pre-B	C/T	T/T

*No. 6,15,24,41 and 42 were relapse cases; No. 43 and 48 shows t(4;11); No. 46 and 47 shows t(9;22)

and C3435T *MDR1* gene polymorphisms can be seen in Table 4. It should be noted that 15 of 22 cases of T-cell ALL were high-risk cases. Among these, 14 of 15 (93%) had C3435T *MDR1* gene polymorphisms, while 12 of 15 (80%) harbored C1236T *MDR1* gene polymorphisms. Notably, 5 of 15 (33%) of the high-risk group were relapse cases (4 cases of Pre-B and one case of Early-pre-B ALL), and exhibited C1236T *MDR1* gene polymorphism, whereas 3 of the 4 relapse cases exhibited C3435T *MDR1* gene polymorphism.

Abnormal cytogenetics was found in 4 cases (27%) from the high-risk group. Two cases had a t(4;11) (q21;q23), which is MLL-AF4 fusion, indicating poor prognosis, and carried C3435T and C1236T *MDR1* gene polymorphisms. The other two cases had t(9;22) (q34;q11), which is BCR-ABL fusion, which also implies poor prognosis, and showed only C1236T *MDR1* gene polymorphism.

Discussion

The multi-drug resistance 1 (*MDR1* or ABCB1) gene encodes a 170-kDa membrane transport protein called P-glycoprotein, which acts as an ATP-dependent exporter of xenobiotics from cells and as an efflux transporter conferring resistance to a variety of natural cytotoxic drugs (Borst and Elferink 2002; Ambudkar et al., 2003). It has been suggested that alteration of the cellular defense mechanism mediated by P-gp is closely associated with the development of various cancers, including breast cancer (Wang et al., 2012), colorectal carcinoma (He et al., 2013; Kurzawski et al., 2005), and ALL (Jamroziak and Rodak, 2004; Sheng et al., 2012). Over the past four decades, the treatment of ALL among children has improved dramatically. Despite this success, drug resistance, and treatment failure due to treatment-related toxicity, still occur in about 20% of patients. A major problem of cytotoxic drug treatment is intrinsic or acquired drug resistance. One potential mechanism of drug resistance is mediated through the expression of the P-gp efflux pump, enabling ALL blasts to decrease intracellular toxic drug levels and thereby lower rates of apoptosis (Hoffmeyer et al., 2000).

Multi-drug resistance (MDR) remains an important challenge during the treatment of acute leukemia. The P-gp function is attributable to the presence of variant *MDR1* genotypes among ALL patients. These polymorphisms could not only influence the sensitivity or resistance of the leukemic blast, but could also impact therapy outcome by altered drug clearance. It has been reported that genetic polymorphisms of the *MDR1* gene may affect the expression and function of the P-gp efflux pump in healthy volunteers (Hitzl et al., 2001 ; Nakamura et al., 2002). At least 105 variants have been discovered in the *MDR1* gene to date. The majority of these SNPs were either intronic or noncoding SNPs, and as such do not change P-gp amino acid composition. Recently, it was demonstrated that the novel c4125 A>C polymorphism of *MDR1* is associated with susceptibility to hepatocellular carcinoma in Chinese population (Ren et al., 2012) but there still not known the role of this novel polymorphism. However, the first *MDR1* SNP reported to be associated with an alteration of P-gp transport function was the silent mutation 3435C→T in exon 26 (Komar, 2007). Studies from cell lines and patient samples have suggested that this polymorphism leads to several changes, from mRNA level, protein expression and protein folding, to substrate specificity (Fung and Gottlesman, 2009).

In this study, C3435T and C1236T *MDR1* polymorphisms were not significantly associated with the development of ALL in Thai children. This observation supports previous reports of childhood ALL in Hispanic (Urayama et al., 2007), Mexican (Leal-Uqarte et al., 2007), Hungarian (Semsi et al., 2008), Chinese (Zhai et al., 2012) and Latvia population (Kreile M et al., 2014). On the other hand, many lines of evidence from Poland (Jamroziak et al., 2004), Japan (Hatori et al., 2007), India (Rao et al., 2010), and Turkey (Bekas-kayan et al., 2012), indicate that these genotypes are involved in the risk of childhood ALL. This genetic variation between different populations of ALL patients may be due to ethnic group differences, sample size, and heterogeneity of ALL patients. Likewise, ALL childhood patients were classified by risk-based assignment protocol into standard and high-risk groups (Smith et al., 1996). This study indicates that both C3435T and C1236T *MDR1* genotypes are significantly associated with high-risk groups for childhood ALL. Moreover, in five relapse cases of ALL were associated with polymorphism of *MDR1* C3435T and C1236T polymorphisms. However, it will be necessary to replicate these finding in large sample size.

In conclusion, *MDR1* C3435T and C1236T polymorphisms are significantly associated with high risk ALL patients, and may be used as potential biomarkers for the prediction of clinical outcomes in childhood ALL.

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